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UNIVERSITY OF CALIFORNIA

Irvine

Mechanical Properties of a Muscle That Determine Power Output: Studies on a Locust Wing Muscle

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biological Sciences

by

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1989

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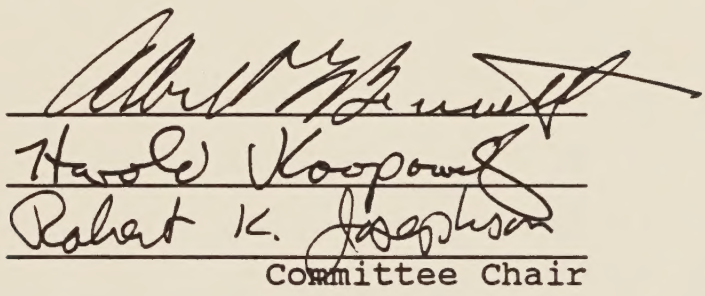

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1989

DEDICATION

This dissertation is dedicated to

Carl, Yvette, and Bruce,

whose encouragement and advice

helped me over the rough spots.

CONTENTS

List of Tables..... vi

List of Figures..... vii

Acknowledgments..... ix

Curriculum Vitae..... x

Abstract..... xi

Chapter 1: The Effects of Octopamine on Contraction Kinetics of a Locust Flight Muscle..... 1

Introduction..... 1

Materials and Methods..... 2

Results..... 8

Discussion..... 21

Summary..... 30

Chapter 2: The Tension in a Locust Flight Muscle at Varied Muscle Lengths..... 31

Introduction..... 31

Materials and Methods..... 33

Results..... 38

Discussion..... 53

Summary..... 61

Chapter 3: Force Velocity Relationships at Different Times During Twitch Contractions..... 62

Introduction..... 62

Materials and Methods..... 64

Results..... 70

Discussion..... 98

Summary..... 106

Appendix: Predicting the Intercept With the Force Axis of a Force-velocity Curve From the Velocity Measured at a Single Force..... 108

References..... 110

LIST OF TABLES

Table		Page
1.1.	The Effects of OA on Twitch and Tetanic Tension of the Locust Tcx_2	10
1.2.	The Influence of Phentolamine on the Response to OA	16
1.3.	Maximum Shortening Velocity is Unchanged by OA	20
1.4.	Twitch Time Course Changes in OA	23
2.1.	Time and Force Constants of the Exponential Function Fitting the Decline in Passive Tension.	43
2.2	Twitch Tension and Twitch/Tetanic Force as a Function of Muscle Length	50
3.1.	Changing Force-velocity Relations During a Twitch Contraction	80

LIST OF FIGURES

Figure		Page
1.1.	The Effects of OA on Twitch and Tetanic Contractions of the Locust Tcx_2.....	9
1.2.	Twitch Tension During Prolonged OA Superfusion.	11
1.3.	Twitch Tension During Repeated OA Application..	12
1.4.	Twitch Tension at Varied OA Concentrations.....	14
1.5.	Dose-response Relation of Tension Enhancement..	15
1.6.	Tension Redevelopment Following a Quick-release	18
1.7.	Maximum Shortening Velocity by the Slack-test..	19
1.8.	Force-velocity Relations in Saline and in OA...	22
1.9.	The Effects of OA on Twitch Contractions of Different Locust Muscles.....	26
2.1.	Passive Muscle Force Following Stretch to Varied Muscle Lengths.....	40
2.2.	Decline of Passive Force Fit to a Multi-exponential Curve.....	42
2.3.	Passive Force as a Function of Muscle Length...	46
2.4.	Active and Passive Force as a Function of Muscle Length in One Muscle.....	47
2.5.	Tetanic Force As a Function of Muscle Length...	48
2.6.	Twitch Contractions at Lo and 126% Lo.....	51
2.7.	Twitch Contraction and Relaxation Times as a Function of Muscle Length.....	52
2.8.	Active and Passive Force as a Function of Muscle Length in Frog and Locust Muscle.....	60
3.1.	Muscle Shortening Following Release to Constant Loads in Twitch and Tetanic Contractions.....	69
3.2.	Force-velocity Relationship in Tetanic Contractions.....	72
3.3.	Isotonic Contractions Early and Late in a Twitch	75

Figure Page

3.4. Shortening Velocity as a Function of Muscle Load at Different Times During a Twitch..... 77

3.5. Normalized Force-velocity Relationships at Different Times During a Twitch..... 79

3.6. Isotonic Shortening at the Same Load at Different Times During the Twitch..... 83

3.7. Relative Velocities at the Same Load at Different Times During the Twitch..... 84

3.8. SA During Twitch Contractions..... 88

3.9. Isotonic Shortening at the Same Load at Different Times During 2 Partially Fused Twitches..... 89

3.10. Tension During Single and Paired Twitches..... 91

3.11. Tension and SA During Paired Twitches..... 92

3.12. Rise and Decline of SA During Twitches..... 93

3.13. Progressive Fusion of Twitch Pairs..... 96

3.14. SA and Active State During a Twitch..... 97

3.15. Shortening Velocity as a Function of Muscle Load and Time During a Twitch..... 100

3.16. Projected Velocity Surface During a Twitch.... 101

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PUBLICATIONS

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Malamud, J.G. (1989). The tension in a locust flight muscle at varied muscle lengths. Journal of Experimental Biology (In Press).

ABSTRACT OF THE DISSERTATION

Mechanical Properties of a Muscle That Determine Power Output: Studies on a Locust Wing Muscle

by

Jean Goldstein Malamud

Doctor of Philosophy in Biological Sciences

University of California, Irvine, 1989

Professor Robert K. Josephson, Chair

Contraction dynamics and length-tension characteristics of a locust flight muscle were investigated in order to characterize the active and passive mechanical properties that influence work and power output. The muscle examined was the metathoracic, second tergocoxal (Tcx_2), a wing levator and coxal remoter, of the locust Schistocerca.

Superfusion of the Tcx_2 by octopamine, a neurohormone, increased twitch and tetanic tension and twitch duration but did not change the maximum shortening velocity (Vmax). At physiological concentrations (10^{-8} to 10^{-5} M) the increase in twitch tension was dose dependent. At 10^{-6} M, twitch tension was potentiated by 19%, tetanic tension by 8%, and twitch contraction and relaxation times were increased by about 4%.

Tension in the unstimulated Tcx_2 increased with increasing muscle length. If the muscle was held at a stretched length, tension declined with time. A multi-exponential function with 4 or more time constants was required to describe adequately the time course of tension decline. The active tetanic tension was maximum at a muscle length slightly less than the in vivo length, while twitch tension was maximal at slightly longer than the in vivo length. Twitch relaxation time, but not contraction time, increased with muscle length in a stretched muscle.

The effect of muscle force on shortening velocity was determined at different times during twitch contractions. Early in a twitch, the values for Vmax and the curvature of the force-velocity relationship were similar to those during a tetanic contraction, but the maximum force (at 0 velocity) was less, and became progressively more displaced with time toward lower forces. Later in the twitch Vmax declined.

A parameter termed State of Activation (SA) is proposed as a measure of the force generating capacity of a muscle. During a twitch, SA rises to a maximum in 2-3 ms after the end of the latent period. The peak value of SA during a single twitch is about 80% of the tetanic value; during the second twitch of a partially fused pair the peak value of SA is similar to that during tetanic stimulation. After a brief plateau, SA declines approximately exponentially with a time constant of about 14 ms at T = 25 ^{o}C.

CHAPTER 1

THE EFFECTS OF OCTOPAMINE ON CONTRACTION KINETICS OF A LOCUST FLIGHT MUSCLE

INTRODUCTION

Octopamine (OA) is a biogenic amine that acts as a local and circulatory neurohormone in many arthropods (Evans, 1981). OA has been shown to potentiate muscle contraction in Limulus, Crustacea, and insects (Rane et al. 1984; Breen & Atwood, 1983; Yoshino & Hisada, 1984; Fischer & Florey, 1983; Candy, 1978; O'Shea & Evans, 1979; Hoyle, 1984).

OA appears to serve a hormonal arousal function similar to that which adrenaline performs in vertebrates -- preparing the animal for fight or flight (Evans, 1980; Orchard et al. 1981). In the cockroach, Periplaneta americana, and the locusts, Schistocerca americana gregaria and Locusta migratoria, the OA concentration in the hemolymph increases during handling or when the animal is subjected to noxious chemical or thermal stimuli (Orchard et al. 1981; Davenport & Evans, 1984). In the cockroach, after one minute of flight there is a peak in the hemolymph OA concentration (6.5×10^{-8} M) that is equal to about twice the resting value (Bailey et al. 1983). In the locust, the OA concentration of the hemolymph increases 5-fold within the first 5 minutes of flight (Goosey & Candy, 1980), reaching 2 x

10^{-7} M before slowly declining to rest values after an hour's continued flight.

OA has a regulatory effect on both energy metabolism and muscle contraction. In locust thoracic preparations, as little as 3 x 10^{-7} M OA enhanced carbohydrate and lipid metabolism in stimulated muscles, while slightly higher concentrations (1 X 10^{-6} M and above) also increased the summed force generated by the activation of the dorsal-ventral muscles (Candy, 1978). In Candy's experiments the relation of OA concentration to increased force was unclear. There was no effect of OA at concentrations of less than 0.3 x 10^{-6} M, and the increase of tension was not monotonically related to OA concentration in the range 1 x 10^{-6} to 5 X 10^{-4} M (Candy, 1978).

It has recently been shown that OA augments the mechanical power output of a locust flight muscle by almost 20% (Mizisin, 1984). The present study was undertaken to better understand that modulation of muscle work performance, by determining the effects of OA on the isometric tension and force-velocity relations of muscle contraction, and by determining the dose-response relation of the OA-mediated enhancement of muscle contraction.

MATERIALS AND METHODS

Experiments were done with muscles of adult, male Schistocerca americana gregaria (formerly S. gregaria) and

S. a. americana. The S. a. gregaria, which were the sub-species used in most experiments, were in the gregarious phase. They were maintained at 26 °C, 55% relative humidity, and a 12 hour light:12 hour dark cycle. Animals were fed bran, skim milk and growing wheat seedlings. S. a. americana were used in measurements of the time course and repeatability of the octopamine effect. S. a. americana is found in Central and North America. It is slightly smaller than the desert locust, but like S. a. gregaria, it is a strong flier (Dirsh, 1974). S. a. americana were raised at 28 °C, 50% relative humidity and a 16 hour light:8 hour dark cycle. They were fed growing wheat seedlings and a commercial soy and beef based dog food.

All experiments were done on in vivo muscle preparations at 30 °C, which is within the normal range of locust body temperatures during flight (Weis-Fogh, 1956). Semi-intact (eviscerated) animals were perfused with physiological saline or saline to which OA or phentolamine (an OA blocker, generously donated by CIBA Pharmaceuticals Co) had been added. Phentolamine was chosen because it is one of the most effective blockers of a wide range of OA-induced responses in insects (Harmar & Horn, 1977; Evans, 1980; Orchard et al. 1981). OA and phentolamine solutions were made up fresh before each experiment. A polyethylene perfusion cannula was fixed in the distal abdomen. Perfusion fluid flowed through the abdomen and thorax and out through a slit in the animal's neck. The saline used was that of

Usherwood & Grundfest (1965), with the addition of 90 mM sucrose (Evans, 1981). An in-line heater, inserted in the polyethylene perfusion tubing, warmed the perfusate to about 28 ^{o}C just before the solution entered the abdomen. The heater was a 2 cm segment of glass capillary tubing wrapped with Nichrome wire that was connected to a low voltage source. Warming the perfusate made it easier to maintain the thoracic temperature at 30 ^{o}C.

The metathoracic second tergocoxal muscle (Tcx_2) was used in all experiments. It is a coxal remoter and an indirect wing levator. The muscle is made up of 3 motor units, served by fast axons running in nerve 4 (Kutsch & Usherwood, 1970; Hedwig & Pearson, 1984). No inhibitory axons appear to innervate this muscle (Hale & Burrows, 1985). The muscle was stimulated with 0.5 ms electrical shocks delivered through electrodes (50 um bare silver wires) implanted on each side of the origin of the Tcx_2. Although shocks were applied directly to the Tcx_2, the muscle was activated by stimulation of the branches of the motor nerves running within it, as evidenced by the three distinct twitch tension increments obtained with sequential stimuli of slowly increasing intensity. The 3 tension increments correspond to the 3 motor units which form the muscle (Josephson, 1973). Stimuli were applied at twice the minimum voltage needed to activate all 3 motor units. Fusion frequency for the Tcx_2 at 30 ^{o}C is about 150 Hz. Tetani were induced with multiple stimuli at a stimu-

lation frequency of 250 Hz. Tetanizing stimulus bursts lasted 50 ms.

The animal was mounted, the preparation perfused, the muscle attached to a force transducer, the muscle temperature monitored and controlled, and the muscle mass and area determined essentially as is described in Mizisin & Josephson (1987).

Muscle force in isometric twitch and tetanic contractions, and muscle force and length change in force-clamp and slack-test experiments, were measured with an ergometer (Cambridge Model 300 H). In force-clamp experiments, the speed of muscle shortening as a function of stress was measured using after-loaded, isotonic, tetanic contractions (Fig. 1.8). A rectangular hyperbola (Hill, 1938) was fit to shortening velocities using the method of Edman et al. (1976). It has been shown by Edman and others that the fit between experimental data and a hyperbola is improved through most of the force range if data at high forces are excluded. Therefore, only data at forces less than 0.8 of the maximum contractile force were used in fitting the curve.

Unloaded shortening velocity (Vo) was measured using the slack-test method, as detailed by Edman (1979). In this technique, a fully active muscle in an isometric tetanus is released to a shorter length, far enough and rapidly enough that its tension drops to zero. There is a time lag before tension redevelops (see Fig. 1.6), whose

duration is a function of the distance of allowed shortening. When release distance is plotted against time to the onset of force redevelopment, a straight line is obtained, the slope of which is Vo. For each Vo series, six releases, ranging from approximately 5% to 15% of muscle length, were applied; and regression lines were calculated for the data by the least squares method. The data for each series fit a straight line with little scatter. All correlation coefficients, r, were 0.99 or higher.

Evans (1981) reported that pre-experimental stress on locusts could affect contraction of the extensor tibia muscle in the same way as did superfusion by OA. Evans found that phentolamine superfusion, followed by a saline rinse, reversed the potentiating effects of exogenous OA which had been previously applied or the potentiation found in initially `stressed' animals. In the experiments described below, except those of Figs. 1.2 and 1.3, a 10^{-6} M phentolamine rinse (5-10 minutes) was applied before each saline superfusion. This was done to minimize any residual effects of OA during saline evaluations. The typical perfusion sequence, therefore, was phentolamine-saline, saline, OA-saline, phentolamine-saline, saline.

After the animal was mounted and saline perfusion begun, regular muscle stimulation with pairs of shocks or tetanic bursts continued throughout the experiment (at one-minute intervals) in order to maintain the muscle in a steady state condition. Paired stimuli were used to avoid

problems introduced by multiple firing following shocks. A stimulus given after tens of seconds of rest sometimes evoked multiple firing of motor units, as evidenced by multiple peaks in the tension response. In twitch measurements, errors due to multiple firing were largely avoided by using pairs of stimuli at one second intervals and measuring the twitch evoked by the second shock of the pair.

Tabulated twitch parameters were taken after 13 to 15 minutes in the specified solutions, except for the first saline solution in which twitches were measured after 15 minutes or longer if the muscle twitches took longer to reach a steady state. In experiments in which parameters of tetanic contractions were determined (maximum isometric tension and force-velocity relations), measurements were collected immediately after the twitch measurements, i.e. after more than 14 to 16 minutes of exposure to the test solution.

Each muscle parameter was measured in saline, then OA-saline, then again in saline. The effect due to OA was calculated as the difference between the OA value and the average of pre-OA and post-OA saline values, with one exception. The exception was one dose-response curve in which there was progressive fatigue of the muscle. In that experiment, a linear regression line was fit to the series of individual twitch tensions in the saline before each OA exposure plotted as a function of time. The twitch tension for each OA challenge was then compared to the calculated

value for tension in saline at that time based on the regression line for the pre-OA period.

RESULTS

Twitch and tetanic tension

Isometric twitch tension increased within 2 minutes of OA exposure. The twitch tension of the Tcx_2 increased an average of 19% over control values (Fig. 1.1 A, Table 1.1) when the muscle was superfused by 10^{-6} M OA. The increase in tension was quite variable in the animals examined, ranging from 3% (0.55 N/cm^2) to 69% (1.6 N/cm^2). The twitch tension reached a maximum after 10-15 minutes of octopamine superfusion, and maintained a plateau for 10 or more minutes (Fig. 1.2).

Enhanced contractile force persisted for some time after a return to normal saline (Fig. 1.2). Persistence of the octopamine effect after return to normal saline superfusion has also been noted in the extensor tibia muscle of the locust (O'Shea & Evans, 1979; Evans & Siegler, 1982) and of the weta (a primitive orthopteran, Hoyle, 1984). For this reason, our normal experimental protocol included a post-octopamine blocker rinse, as explained in the materials and methods section. Repeated exposures to octopamine, separated by phentolamine and saline rinses, gave repeated augmentation of twitch tension (Figs. 1.3 and 1.4).

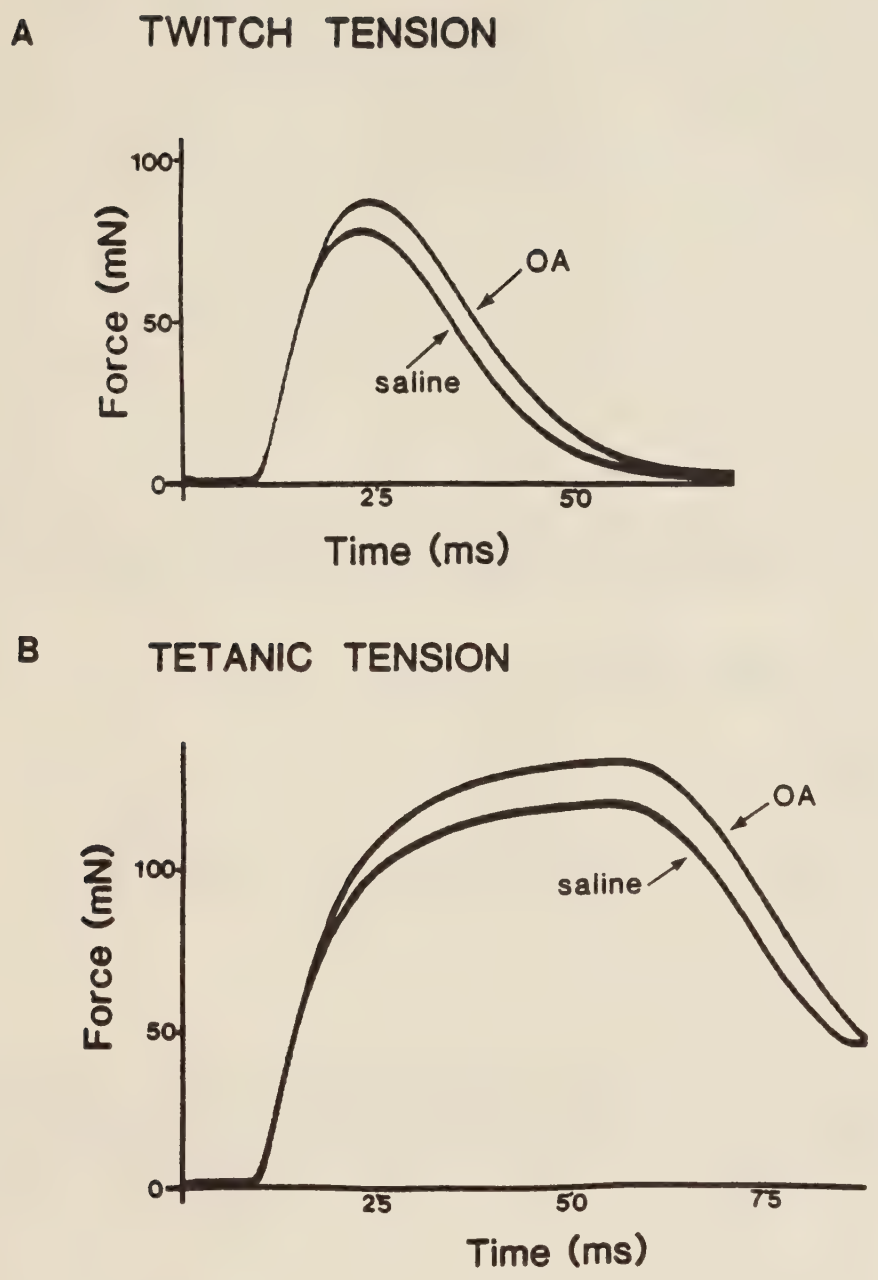

Fig. 1.1. Twitch and tetanic tensions in saline and in saline containing 10^{-6} M OA, 30 ^{o}C.
A. Isometric twitch contractions.
B. Isometric tetanic contractions (stimulation frequency = 250 Hz). The increase in tetanic tension in this example was one of the highest measured (11.4%), while the twitch tension enhancement (12.5%) was somewhat less than average.

Table 1.1 Isometric twitch and tetanic tension produced by the Tcx_2 at 30^{o} C when bathed by saline or by 10^{-6} M OA

	TENSION (N/cm^2, mean ± s.e.)	
	TWITCH (n=14)	TETANIC (n=12)
Saline		
pre-OA	17.2 ± 1.2	29.5 ± 2.3
post-OA	16.8 ± 1.1	26.2 ± 1.7
OA	*20.3 ± 1.3	*30.0 ± 1.8

* Statistically significant, $p < 0.001$ (Student's t-test, paired samples comparing OA response with the mean of the pre- and post-OA saline responses). The standard errors of the paired differences were 0.7 for twitch and 0.3 for tetanic responses.

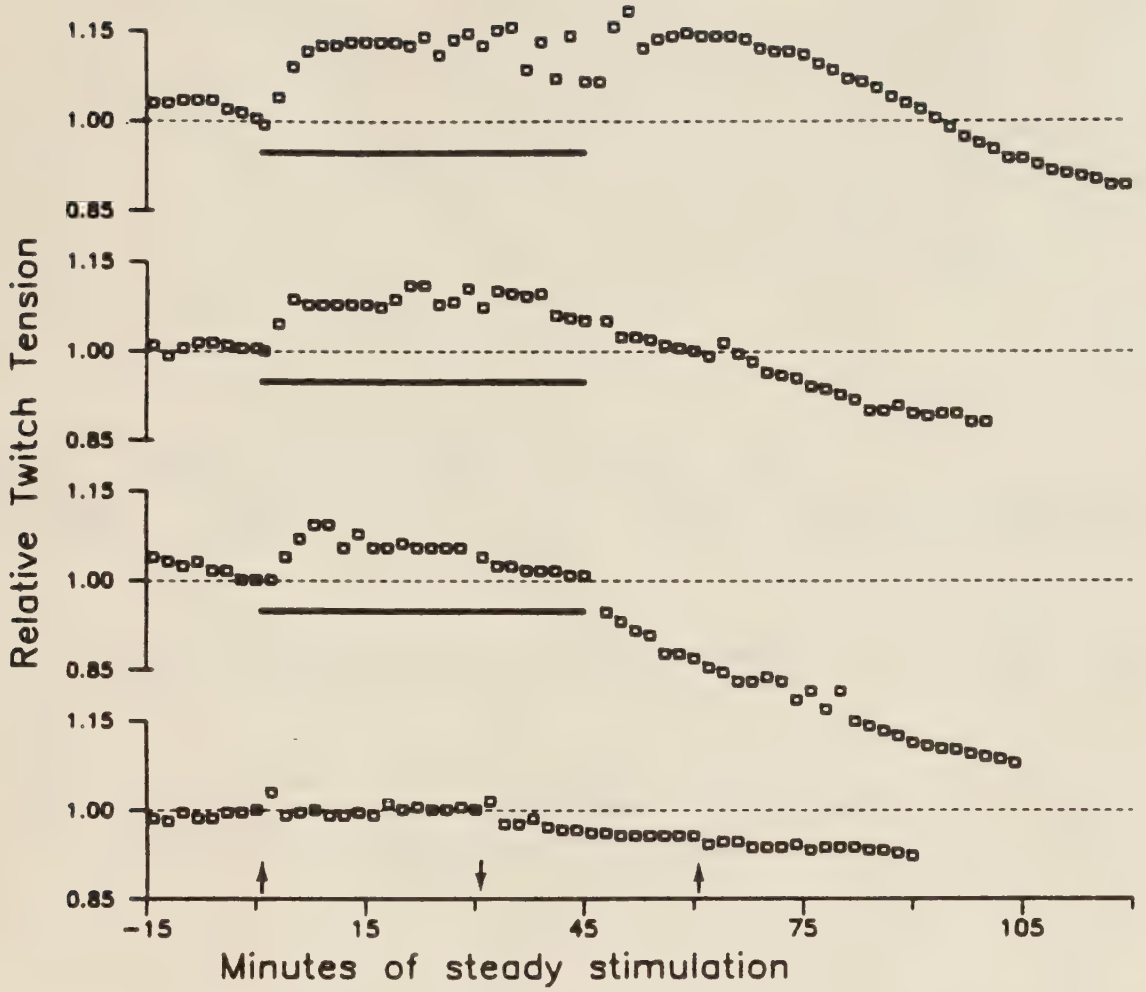

Fig. 1.2. Twitch tension during muscle superfusion by saline, 10^{-6} M OA, and again by saline. The period of OA superfusion is marked by a horizontal bar. The muscles were stimulated at one-minute intervals. In order to simplify presentation, only the results from every other minute are plotted. In the lower record the second perfusate source, that which normally contained OA, contained saline alone. Up arrows mark switch of the perfusate to this source, down arrows a return to the original source. Trials like those in the upper data sets were done with 5 preparations, 3 of which are plotted. One of the others was inadvertently terminated prematurely and in the last the twitch tension from trial to trial was extremely and erratically variable. In these trials and in others with S. a. americana the average increase in twitch amplitude in 10^{-6} M OA was 14.4% (n=7) which is somewhat less than the increase recorded with S. a. gregaria, and which may reflect a sub-species difference.

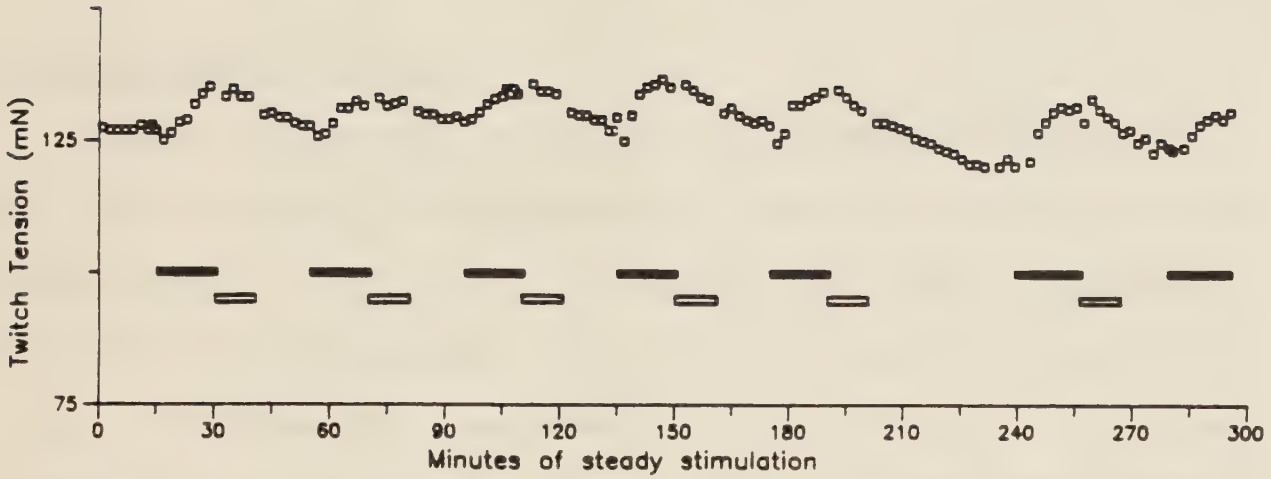

Fig. 1.3. Twitch tension during repeated application of 10^{-6} M OA (marked by solid bars). The trials shown are from preparation `c' of Table 1.4. The muscle was stimulated at one-minute intervals. In order to simplify presentation, only the results from every other minute are plotted. For the first OA period, the muscle had been superfused by nothing but saline. OA periods 2 through 5 followed 10 minutes of 10^{-6} M phentolamine superfusion (marked by open bars) and 15 minutes of saline (mean $\pm$ s.d. = 135 mN $\pm$ 1.8, n = 4). Note that after a prolonged period of saline perfusion, the muscle again responded with approximately the same twitch force when exposed to 10^{-6} M OA and then by the same OA solution following phentolamine exposure and washout (trial 6 = 132 mN; trial 7 = 131 mN).

There was a moderate increase (average 7.7%) in maximum isometric tension (Po) in muscles superfused with 10^{-6} M OA (Fig 1.1 B, Table 1.1). As with the twitch tension, there was a wide variability in tetanic force augmentation brought about by octopamine. Tension enhancement ranged from 0.65 N/cm^2 (2% over saline) to 3.5 N/cm^2 (13% increase) in the muscles examined.

Dose-response relation

Twitch tension was measured in OA concentrations of 10^{-8} M to 10^{-4} M. Each muscle was tested with a series of solutions at increasing OA concentration, with intervening exposure to phentolamine and normal saline solutions (Fig. 1.4). Twitches were measured after 15 minutes in OA solution, or during the plateau of effect if this came earlier. The fractional enhancement of twitch tension by OA was approximately proportional to the log of OA concentration over a 3 decade concentration range (Fig. 1.5).

Dose-response curves determined by repeated application of drugs and antagonists to the same preparations, as was done in the experiments that led to Figures 1.4 and 1.5, could be affected by cumulative effects of either drug or antagonist. There do not appear to be significant cumulative drug effects on the locust muscle with the protocol used. Twitch tension was measured in 3 preparations during an initial superfusion by 10^{-6} M OA, then again two or more times after phentolamine exposure. The muscle response to

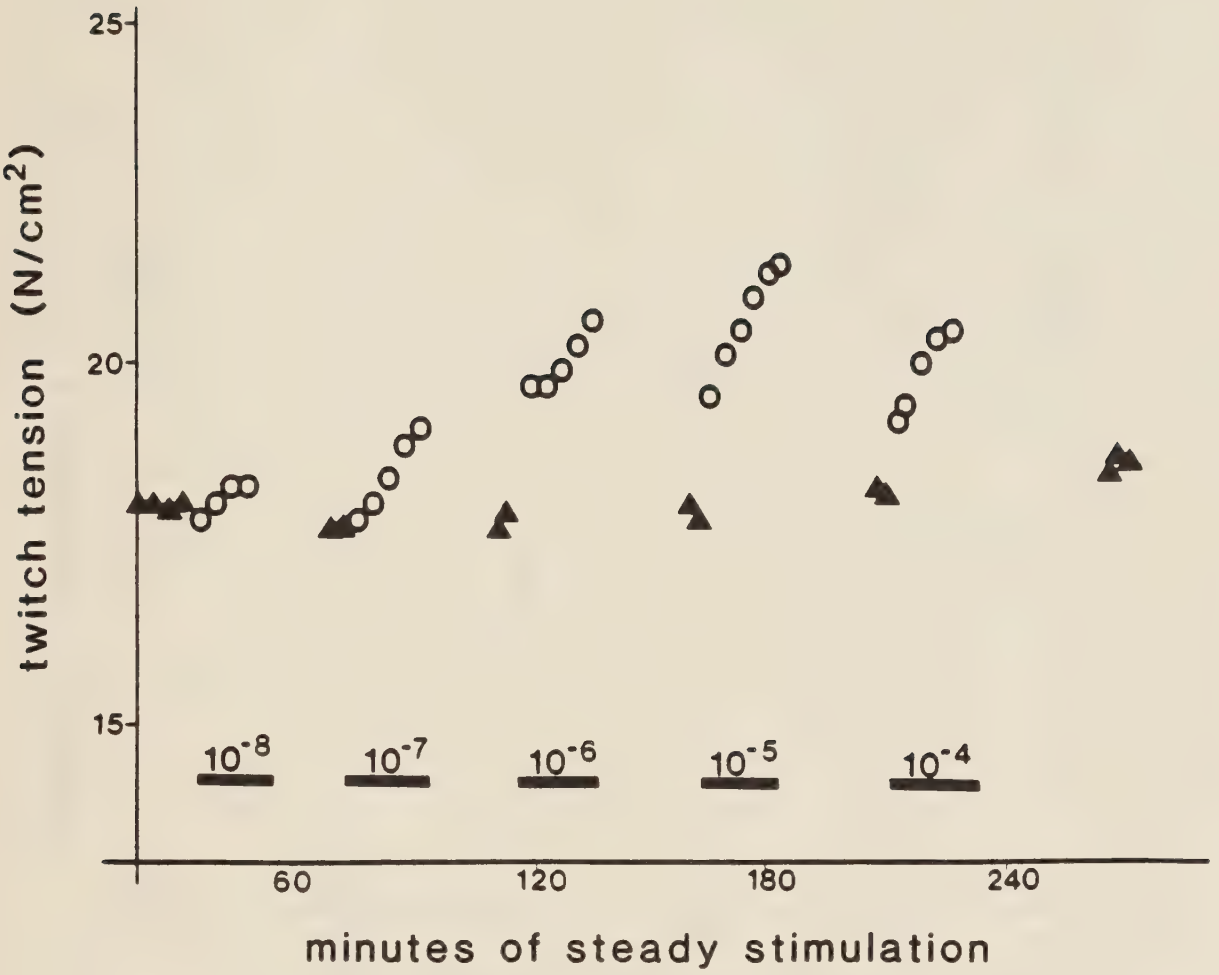

Fig. 1.4. Twitch tension in saline (triangles), and in saline with OA (circles). The OA periods are marked by bars, with the OA concentration (M/l) indicated above each bar. The OA challenges were separated by a wash-out in phentolamine (10 minutes) and then saline. The muscle temperature was allowed to fall below 30 ^{o}C during the phentolamine rinse, and although stimulation continued at one minute intervals, twitch tension was not recorded. The muscle was rewarmed during the saline rinses and maintained warm during OA application. The tensions of the two twitches in saline just before each OA application are plotted. In order to simplify the presentation, only the tension of every second or third trial is shown for the periods in which the muscle was in OA solution.

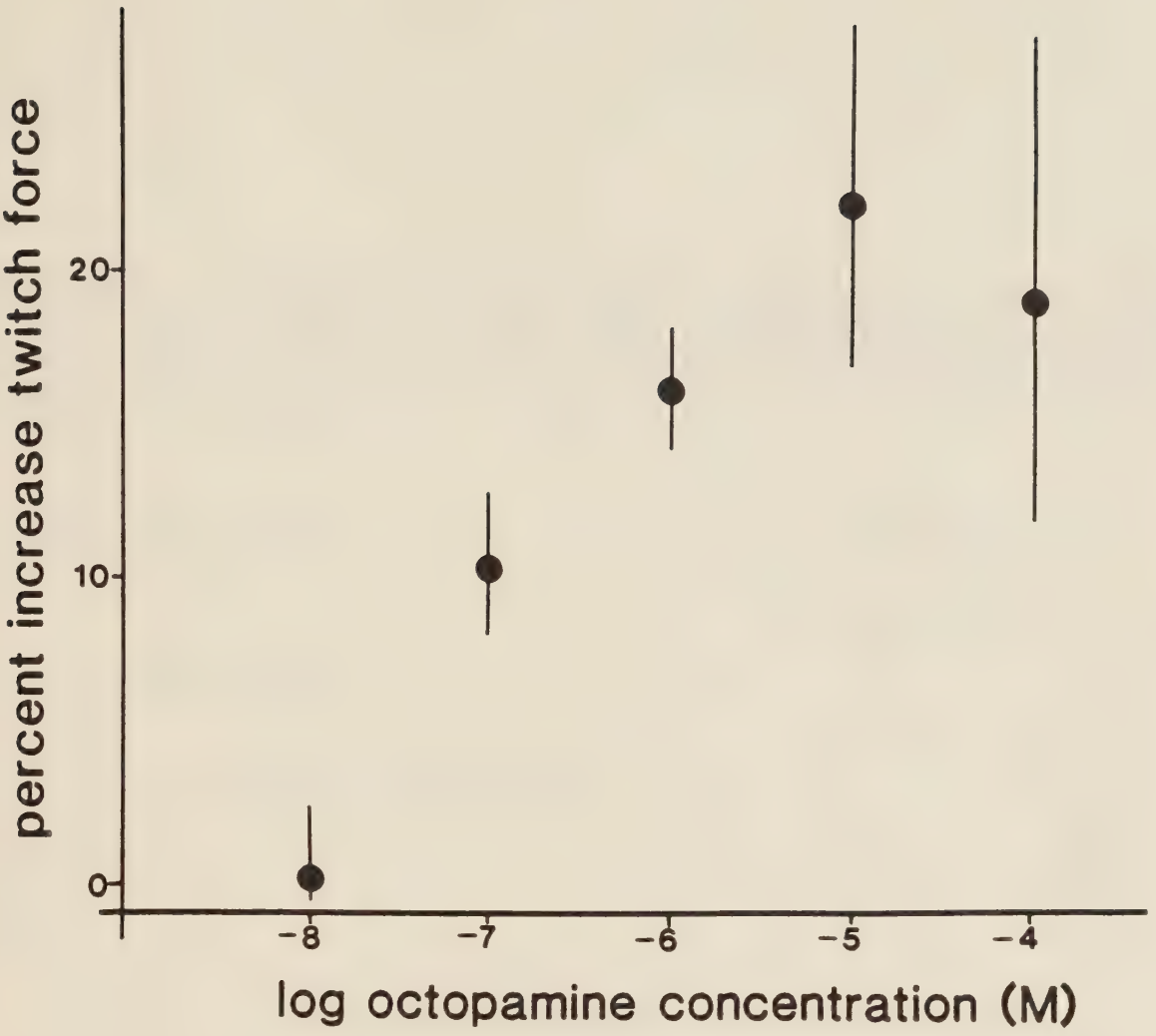

Fig. 1.5. Enhancement of twitch tension as a function of OA concentration. Points are the means of determinations on 3 preparations and the vertical bars represent standard errors. Both means and standard errors were determined using an arcsine transformation.

Table 1.2. Isometric twitch tension produced by the Tcx_2 at 30 ^{o}C when bathed by 10^{-6} M OA during first superfusion by other than saline, and by 10^{-6} M OA after exposure to 10 minutes of phentolamine followed by 15 minutes of saline.

PERFUSION ORDER	TWITCH TENSION (mN)		
	Preparation		
	a	b	c
after saline alone:			
OA # 1	118	141	135
after phentolamine and saline:			
OA # 2	121	135	133
OA # 3	123	134	135
OA # 4	---	---	137
OA # 5	---	---	135

OA before any exposure to phentolamine was not significantly different from the responses after phentolamine application and washout (Fig. 1.3, Table 1.2). In addition, in the dose-response series (Fig. 1.5), the average twitch tension increase at 10^{-6} M OA, which was measured after 2 preceding trials at other concentrations, was quite similar to that in other experiments, in which the muscle was exposed only to OA at 10^{-6} M (Table 1.2).

Shortening velocity

Maximum shortening velocities were measured by the slack-test method (Fig. 1.6). Following Edman (1979), these values are identified as Vo. Vo was similar (approximately 8 muscle lengths per second) in solutions with or without OA (e.g. Fig. 1.7). There was, in fact, a slight decrease (3%, $p < 0.05$) in the average Vo with exposure to OA (Table 1.3).

Maximum shortening velocity was also obtained by fitting a hyperbola to force-velocity points collected during after-loaded, isotonic contractions (Fig. 1.8). The hyperbola was of the form $(P+a)(V+b) = (Po+a)b$; where P=force, V=velocity, Po=maximum tetanic tension, and a and b are constants (Hill, 1938). The maximum shortening velocity determined by extrapolation of this force-velocity curve to the velocity axis is termed Vmax. In these experiments, there was a small but not statistically significant

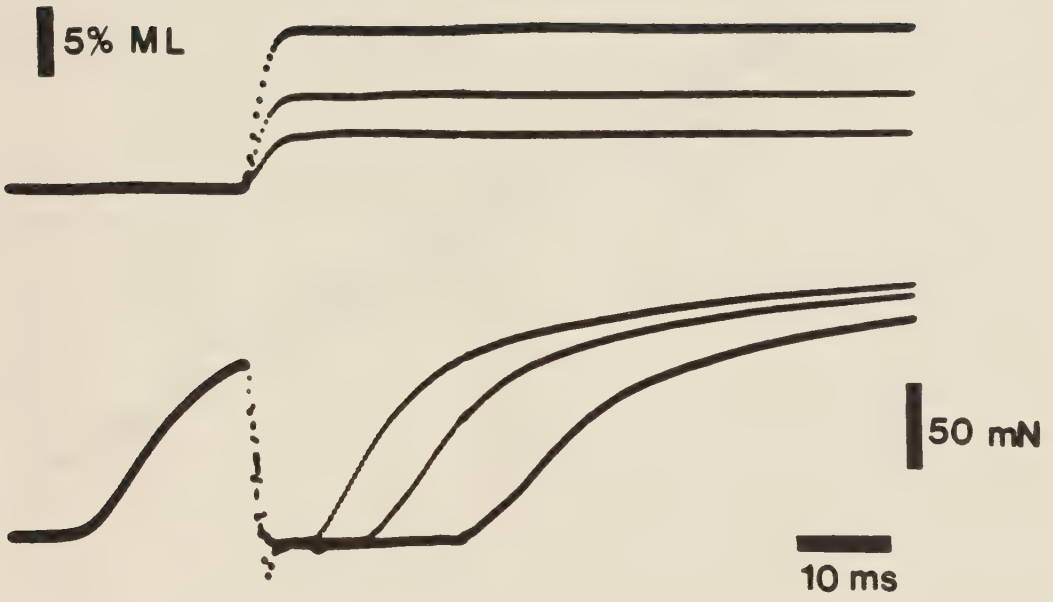

Fig. 1.6. Tension redevelopment following a quick-release. The upper set of traces is muscle length (shortening is an upward deflection). The lower set of traces is force development in the tetanus and redevelopment after the release. The greatest release takes the longest time to redevelop tension. ML = muscle length.

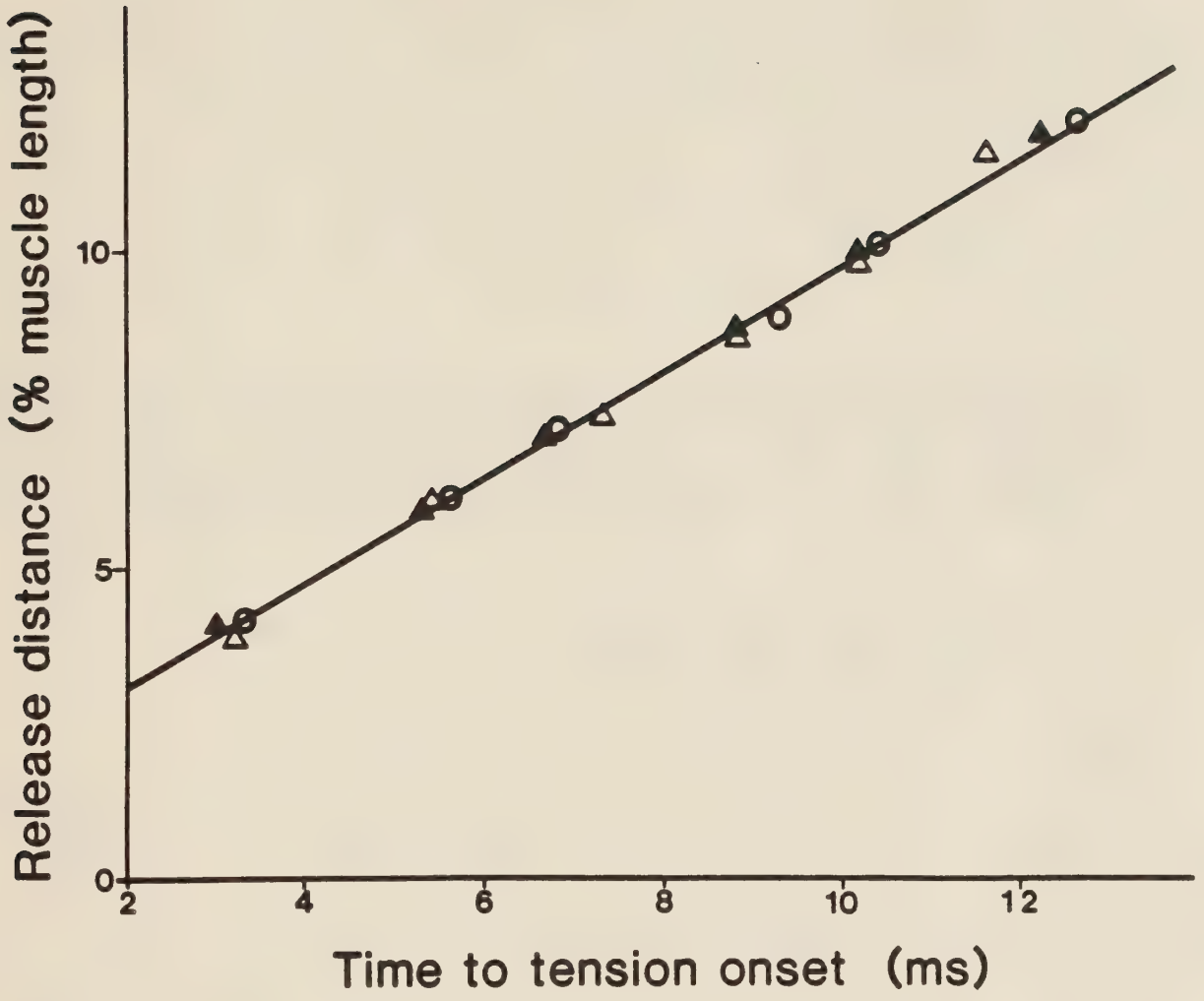

Fig. 1.7. Slack-test measurement of maximum shortening velocity. The delay to the onset of tension redevelopment following quick-release is plotted as a function of release distance. The slope of the line relating release distance and time to tension redevelopment is Vo, the maximum shortening velocity at 0 load. This data is from the same preparation as in Fig. 1.6. Solid triangles are values from muscle in saline before OA application, circles are values from muscle in saline containing 10^{-6} M OA, open triangles are values from muscle in saline after OA exposure. The solid line is a linear regression fit to the points of the OA data.

Table 1.3. Maximum shortening velocity of muscles in saline or in saline containing 10^{-6} M OA. The maximum velocity was determined by the slack-test (Vo) and by extrapolation of force-velocity curves to zero force (Vmax). T=30 °C.

	SHORTENING VELOCITY (ML/s, mean ± s.e.)		
	Vo (n=5)	Vmax (n=7)	a/Po (n=7)
Saline			
pre-OA	8.4 ± 0.2	5.8 ± 0.5	0.25 ± 0.03
post-OA	8.2 ± 0.2	5.5 ± 0.6	0.35 ± 0.03
OA	*8.0 ± 0.2	5.9 ± 0.6	0.33 ± 0.09

* Statistically significant, $p < 0.05$ (Student's t-test, paired samples comparing OA response with the mean of the pre-and post-OA saline responses). ML=muscle length.

increase in Vmax upon exposure of the muscle to OA (Table 1.3).

The curvature of a force-velocity hyperbola is typically measured by the ratio of a and Po from the Hill equation. By this measure, the curvature of the force-velocity relationship was similar in OA and in saline. a/Po was 0.30 (s.e.=0.02) in saline and 0.33 (s.e.=0.09) in 10^{-6} M OA. It should be noted that force-velocity curves for a muscle exposed to or not exposed to OA are essentially identical except at high forces (Fig. 1.8). It is because of this similarity in force-velocity relation at low and intermediate forces that the early tension rise in isometric twitch and tetanus remains unaffected by OA (Fig. 1.1).

Twitch time course

There were some changes in the twitch time course under the influence of OA, but these were relatively small (Table 1.4). The twitch rise time, decay time, and time of half-maximal force duration increased in 10^{-6} M OA, but for each of these parameters the increase was only 4-5%.

DISCUSSION

This study was begun to characterize the effects of octopamine on the contractile performance of locust flight muscle, and thus to identify the mechanisms which might underlie the octopamine-mediated increase in mechanical

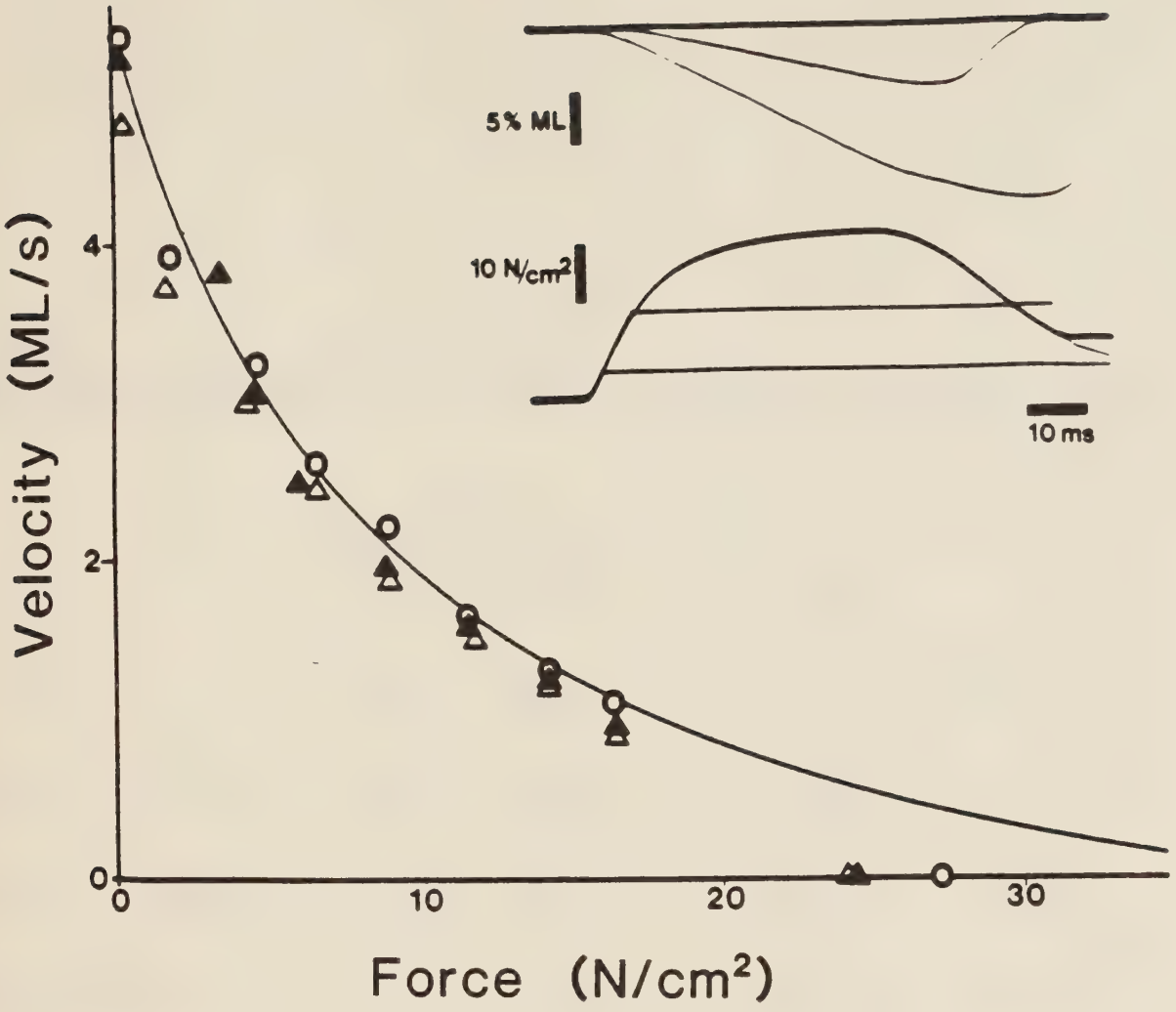

Fig. 1.8. The force-velocity relationship of the Tcx_2 in normal saline and in saline with 10^{-6} M OA. The separate symbols indicate responses in pre-OA saline (solid triangles), post-OA saline (open triangles), and OA (circles). The curve shown is a Hill hyperbola fit to the points from the OA data, excluding the isometric force (the point on the abscissa). INSET: After-loaded, isotonic contractions (stimuli at 250 Hz). The upper set of traces is muscle length (shortening is downward), the lower set is force. The top trace in each set is from an isometric tetanus. ML = muscle length.

Table 1.4. Isometric twitch time course (ms; T=30° C) of muscles superfused with 10^{-6} M OA and with saline. (n=14)

	SALINE		OA	
	pre-OA mean (s.e.)	post-OA mean (s.e.)	mean (s.e.)	p*
Rise time	14.0 (0.4)	14.1 (0.3)	14.7 (0.3)	<0.01
Twitch duration	40.1 (0.9)	40.8 (1.0)	42.2 (0.8)	<0.01
Half relaxation	27.8 (0.9)	28.3 (0.9)	29.1 (0.8)	<0.02
Half-force duration	23.4 (0.6)	23.9 (0.6)	24.5 (0.5)	<0.01

NOTES: * Student's t-test, paired samples comparing the OA response to the average of the pre- and post-OA saline responses. Rise time: onset to twitch peak. Twitch duration: onset to 90% relaxation. Half relaxation: onset to 50% relaxation. Half-force duration: Time at greater than 0.5 peak force.

power output found in this muscle (Mizisin, 1984). Exposing the muscle to exogenous OA increases twitch and tetanic tension and slightly increases the twitch duration, but has little or no effect on the maximum shortening velocity or the curvature of the force-velocity relation (Tables 1.1, 1.3 and 1.4). The increase in maximum isometric tension (8%) and the increase in twitch duration (4%) at 10^{-6} M OA are both considerably smaller than the increase in work output. However, the increase in twitch tension (19%) was quite similar to that of work output found by Mizisin (1984), and is probably a reflection of the same changes within the muscle.

It is surprising that the fractional enhancement of twitch tension upon exposure to OA is about 2.5 times that of tetanic tension (Table 1.1). In the different muscles examined there was a correlation between the fractional enhancement of twitch tension and the fractional increase in tetanic tension in the same muscle (Kendall's coefficient of rank correlation, tau, is significant at the 5% level). An increase in twitch tension could be a result of an increase in the duration of the active state (Ritchie, 1954) or of an upward shift of the hyperbolic force-velocity curve brought about by any combination of (i) an increase in maximum shortening velocity, (ii) an increase in maximum tetanic tension, or (iii) a decrease in the curvature of the hyperbola. As mentioned above, the maximum shortening velocity and the curvature of the force-velocity

relation are not affected by octopamine. Apparently, a small change in the duration of the active state, reflected in an increased twitch duration, and a moderate change in maximum tetanic tension synergistically interact to give a proportionally greater change in twitch tension, and presumably in work output per cycle as well.

One well documented effect of OA on insect muscles is an enhancement of neuromuscular transmission (Evans, 1981 and references therein; Klaassen & Kammer, 1985). The effects of OA on the contractile properties of the Tcx_2 could also be explained by enhanced neuromuscular transmission. More transmitter release would lead to larger excitatory junction potentials all along the muscle fibers, and thus a stronger activation of individual fibers in both twitch and tetanus. Enhanced neuromuscular transmission could also produce a longer period of transmitter release, resulting in the observed slightly longer contraction period and thus a stronger twitch.

The potentiation of contraction by OA is dose dependent and significant at physiological concentrations. In early flight, circulating octopamine titers in both cockroach and locust rise to levels (10^{-7} to 10^{-8} M) that are within the dose-response range for which OA is effective in enhancing twitch force (Fig. 1.5). It should be noted that the dose response curve of Fig. 1.5 is based on responses to DL-OA. Since the naturally occurring stereoisomer in the locust, and the one that is most effective, is D-OA (Goosey &

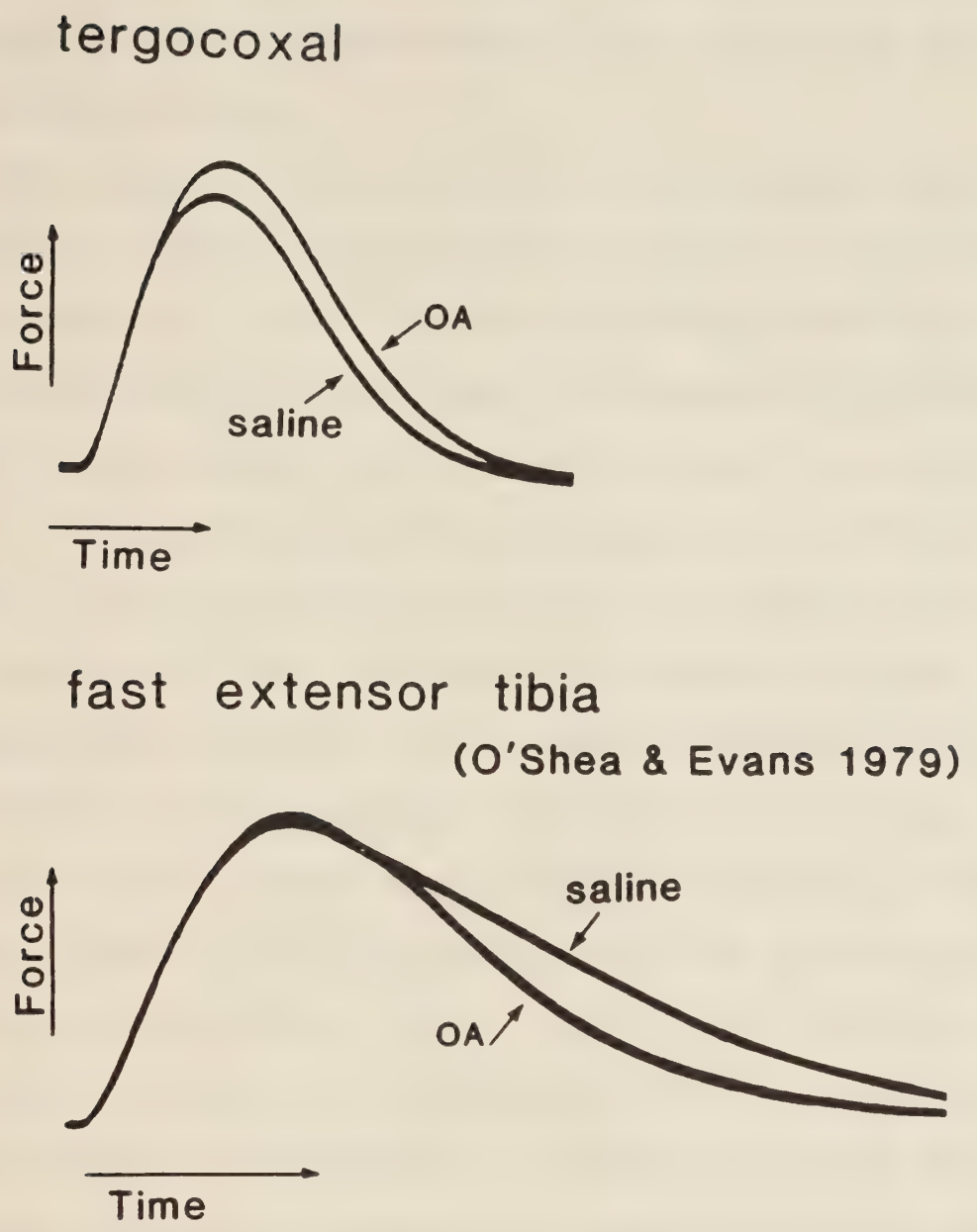

Fig. 1.9. Twitch tension of two locust muscles in saline and in saline containing 10^{-6} M OA. Time and tension for the two muscles are scaled differently in order to compare the different effects of octopamine on the fast extensor tibia (adapted from O'Shea & Evans, 1979) and on the Tcx_2.

Candy, 1980; Evans, 1981), the dose-response curve for the racemic mixture underestimates the true muscle responsiveness to this drug.

The action of OA on the Tcx_2 is different than its effect on another locust muscle, the extensor tibia of the hind leg (Fig. 1.9). OA does not affect the twitch tension of the extensor tibia evoked by stimulation of the fast axon to the muscle, but it does increase the relaxation rate, thus decreasing the twitch duration (O'Shea & Evans, 1979). The differential effects of OA may be related to different ways that leg and wing muscles are used. Leg muscles are used over a wide range of frequencies, from standing to rapid running. At higher frequencies, muscle action is more efficient if muscle relaxation is fully or nearly complete before the onset of the antagonistic (lengthening) half cycle. Thus it may be of functional significance to shorten the twitch duration during intense activity at higher frequencies. In flight, on the other hand, greater locomotory velocity is associated with greater wing-stroke amplitude and angle of attack, and there is little change in wing-stroke frequency (Weis-Fogh, 1956). Thus there is no obvious advantage to shortening twitch durations during vigorous flight.

The changes in maximum shortening velocity in response to OA were small and contradictory. Vmax, the maximum velocity as determined by the extrapolation of a force-velocity curve to zero force, showed a small (not statisti-

cally significant) increase upon exposure to octopamine. The change in Vo, the maximum velocity determined by the slack-test, was a small but significant ($p<0.05$) decrease.

The maximum shortening velocity found by the slack-test was 1.4 times that determined by extrapolation of velocities in after-loaded, isotonic contractions. A difference in maximum velocity determinations by the slack-test and force-clamp methods has been found in both insect and vertebrate whole muscles (Josephson, 1984; Claflin & Faulkner, 1985), and is seen to some extent even in single fibers (Edman, 1979). Vo in whole muscles is a measure of the unloaded shortening velocity of the fastest fibers, while Vmax reflects the force-velocity characteristics of the entire muscle (Claflin & Faulkner, 1985). Vo and Vmax can be expected to be different if there is fiber heterogeneity in the muscle. Finding that there is a difference between Vo and Vmax suggests that there is some heterogeneity in the Tcx_2, even though it is composed of only 3 motor units, the individual twitches of which are all quite similar in time course.

Lately there has been some controversy about whether or not the maximum shortening velocity of muscle changes with the state of muscle activation. Jewell & Wilkie (1960) found that the maximum shortening velocity of frog muscle declined through the time course of the twitch as muscle activation waned. More recently, Cecchi et al. (1983), and Podolin & Ford (1986) found that the maximum shortening

velocity was independent of activation level in intact and skinned frog muscle fibers. In contrast, other reports indicate that both force and speed of shortening are dependent on the state of activation of skinned muscle fibers (Julian & Moss, 1981; Julian et al. 1986). In locust muscle, OA does seem to change the state of activation of the muscle, as evidenced by the increased maximum tetanic tension, but it does not affect maximum shortening velocity. Here, at least within the range of activation provided by differing OA levels, the maximum velocity of contraction would seem to be independent of the state of activation of the muscle.

SUMMARY

Perfusion with saline containing octopamine resulted in an increase in twitch and tetanic tension from a flight muscle (metathoracic, second tergocoxal muscle) of the desert locust, Schistocerca americana gregaria.

The increase in twitch tension was approximately proportional to the log of the octopamine concentration from 10^{-8} M to 10^{-5} M. At 10^{-6} M, the twitch tension was about 19% greater than control values and the tetanic tension was about 8% above control values. The average twitch tension in control muscles was 17.0 N/cm^2, and the average control tetanic tension was 27.9 N/cm^2.

The maximum shortening velocity was little affected by octopamine. In control muscles, the maximum shortening velocity was 8.3 ML/s for slack-test measurements and 5.7 ML/s by extrapolation of force-velocity curves. In octopamine, the corresponding maximum shortening velocities were 8.0 and 5.9 ML/s respectively.

Octopamine perfusion resulted in a small increase in twitch duration (control mean = 40.5 ms at 30 ^{o}C). Both contraction and relaxation times increased by about 4%.

CHAPTER 2

THE TENSION IN A LOCUST FLIGHT MUSCLE AT VARIED MUSCLE LENGTHS

INTRODUCTION

Vertebrate skeletal muscle is cross striated, due to the aligned sarcomeres of the myofibrils. Arthropod muscle is also cross striated, and is similar to vertebrate skeletal muscle in many other aspects of form and function. Insect flight muscle, however, is often described as being unusual, differing from other striated muscles mechanically and physiologically (Podolsky & Schoenberg, 1983; Tregear, 1983; McMahon, 1984; Aidley, 1985). Some insect flight muscles do differ from vertebrate muscles in that the timing of their cyclic contractions is determined largely by mechanical events and not by phasic input from motor neurones. Such muscles were called asynchronous by Pringle (1949) when he found that the muscle contraction frequency was not directly correlated with the motor nerve frequency (for discussions of asynchronous muscles see Pringle, 1981; Tregear, 1983; Aidley, 1985). The asynchronous muscles are unusual in several respects, including their ability to oscillate, and in being reported to be extremely stiff and inextensible (Machin & Pringle, 1959).

Although asynchronous flight muscles are unusual, the flight muscles of many insects are neither asynchronous nor peculiar. Dragonflies, locusts, moths, and insects in sev-

eral other orders have wing muscles with contraction frequency directly controlled by motor nerve firing patterns, as are those of other arthropod and vertebrate skeletal muscles.

The length-tension relations of one major locust flight muscle, the dorsal longitudinal muscle (DLM), have been studied. This muscle was described as being almost as stiff and inextensible as the few asynchronous muscles that have been examined (Weis-Fogh, 1956a). These DLM studies were included in an early review of muscle structure and function (Hanson & Lowy, 1960), and have been extensively reported. It has become generally accepted that insect flight muscles, both synchronous and asynchronous, are very stiff even when relaxed (Buchthal & Weis-Fogh, 1956; Hanson & Lowy, 1960; Tregear, 1983; McMahon, 1984; Aidley, 1985), and that the length-tension relations are unlike those of conventional vertebrate skeletal muscle (i.e., frog leg muscle).

The studies presented in this paper were undertaken to elucidate the length-tension relations in a locust wing muscle. The resistance to stretch that was examined was the low frequency stiffness, similar to that measured in other muscles to establish passive length tension curves. Surprisingly, this muscle was not unusually inextensible, and its length-tension properties, both when stimulated and at rest, were not greatly different from those of frequently-studied vertebrate muscles.

Passive tension in a stretched muscle is a function of time after stretch as well as of the distance stretched. The force in a resting, stretched muscle falls continuously from the value reached at the end of stretch. The decline in stress is called stress relaxation. The extent and time-course of stress relaxation were investigated in order to determine an appropriate time after the end of stretch at which to measure the passive length-tension relations, as well as to obtain some insight into the components that are responsible for the stiffness of the resting muscle.

MATERIALS AND METHODS

Adult, male locusts, Schistocerca americana (Drury), were used in all experiments. They were maintained at 29 ^{o}C, and on a 16 hour light:8 hour dark cycle. Animals were fed growing wheat seedlings and a commercial dog food, based on soy and beef.

All experiments were done on in vivo preparations of the metathoracic second tergocoxal muscle (Tcx_2), which is an indirect wing levator and a coxal remoter. This muscle extends from the tergum to the posterior coxal rim. It is composed of parallel fibers, all of which are nearly the same length. The muscle is approximately 8 mm long and weighs about 4 mg. Locust flight muscle is very sensitive to oxygen lack (Weis-Fogh, 1956a). Therefore care was taken

during muscle preparation to keep intact the tracheae that form the pathway of oxygen delivery to the Tcx_2.

To prepare the muscle for recording, the locust's wings and legs were detached, after which the metathoracic ganglion was cut free and removed in order to avoid spontaneous Tcx_2 activation or extraneous movement. In some stress relaxation experiments, the mesothoracic ganglion was also removed to reduce movement artefacts further. All muscle attachments except those of the Tcx_2 were cut from one metathoracic coxa, and the coxal cuticle was trimmed to a small piece of the proximal rim containing the attachment site of the Tcx_2 apodeme. Silver wire electrodes (50 u diameter) were inserted through holes in the tergum on either side of the origin of the Tcx_2 and waxed in place. The animal was then fastened on its back with epoxy glue to a flat holder.

Muscle force and position changes were measured with an ergometer (Cambridge Model 300 H), to which the muscle was coupled by an insect pin (about 15 mg) that was bent into a hook at each end. The animal on its holder was mounted at 45^{o} on a plastic platform, with the abdomen higher than the head. At this angle the Tcx_2 was vertical, and the muscle force developed was normal to the horizontal transducer arm. The plastic platform was mounted on a micromanipulator so that it could be positioned with respect to the ergometer. At the start of each experiment, the remnant coxal cuticle was slipped over the insect pin hook, linking

the Tcx_2 muscle and the transducer. The Tcx_2 was stretched to approximately its normal *in vivo* length, as judged by the position of the coxal remnant relative to the sternum when viewed through a dissecting microscope. This experimental length was used as reference length (Lref) in all experiments.

The thoracic temperature was monitored by a thermistor inserted in the thorax through the contralateral coxa. The output of the temperature monitor also controlled the intensity of a microscope lamp shining onto the animal's sternum. A decrease in temperature increased the light intensity. In this way the temperature of the thorax was maintained at 25 ^{o}C by feedback control. The muscle was moistened throughout the experiments with locust saline (Usherwood & Grundfest, 1965) or with saline to which 90 mM sucrose had been added (Evans, 1981).

The stimuli for muscle contractions were 0.5 ms electrical shocks through the implanted electrodes. Three distinct twitch tension increments were obtained with sequential stimuli of slowly increasing intensity--corresponding to the 3 motor units which form the muscle (Kutsch & Usherwood, 1970; see Josephson, 1973 for a discussion of relative stimulation thresholds for nerve and muscle). During measurements of twitch or tetanic contractions, the shocks were applied at 1.5 times the minimum voltage needed to activate all 3 motor units. Tetani were induced with multiple stimuli at a frequency of 250 Hz. Tetanizing

stimulus bursts lasted 50 ms. A single stimulus given after tens of seconds of rest sometimes evoked multiple firing of motor units, as evidenced by multiple peaks in the tension response. Multiple firing was rare with inter-stimulus intervals of a second or less. In twitch measurements, errors due to multiple firing were largely avoided by using a pair of stimuli with a 0.5 s interval, and measuring the twitch evoked by the second shock of the pair.

In stress relaxation experiments, a command voltage to the ergometer position control caused a quick change in muscle length. Length changes were at 50 - 80 mm/s. After reaching the new length, the muscle remained stretched for 15 minutes. Force and position values were monitored with an oscilloscope, and collected with A to D converters at 2 ms per sample for the first 1 to 2 s and at 0.13 s per sample for 15 minutes. At the end of a stretch period the muscle length was returned to Lref. At the beginning of an experiment, and after the return to Lref following a stretch, the muscle was stimulated several times with stimulus pairs (0.5 s interval) at 30 s intervals to take up the slack and to monitor potential deterioration of the preparation. This stimulation was followed by more than 2 minutes of rest before the next elongation.

In experiments on the effect of muscle length on passive, twitch, and tetanic tension, the muscle length changes were accomplished in 3 to 5 s by manually adjusting the voltage controlling the position of the ergometer arm.

One minute after the change in length, the muscle was tetanically stimulated. One minute after the tetanic stimulation, a twitch pair was evoked, after which the muscle length was returned to Lref. The same stimulation sequence was repeated at Lref. With this protocol each experimental value was obtained approximately 2 minutes after a corresponding reference value and was followed, after another two minutes, by another determination of a reference value.

All experiments started with 10 to 20 twitch pairs at approximately 30 s intervals at Lref in order to allow the muscle to reach a steady state. After equilibration and testing at Lref, the muscle length was adjusted to the shortest length to be investigated. After testing at this and each subsequent experimental length, the muscle length was again returned to Lref. At Lref, the twitch and tetanic tension were characterized as described above. The experimental lengths examined were progressively greater, and the largest strains were examined last. An increasing length series was used rather than randomized length changes so that long extensions, which can damage the muscle, would not affect the results at shorter lengths. It has often been observed that the force of a twitch following a tetanus is greater than that preceding it, a phenomenon called post-tetanic potentiation. In the locust muscle post-tetanic potentiation persists for some time, 3 - 5 minutes at 25 ^{o}C (unpublished observation). In order to have a constant influence of any post-tetanic potentia-

tion, in investigations including both twitch and tetanus all twitches were one minute after a tetanic contraction.

At the end of each experiment, the muscle was held at Lref and fixed by injecting the thorax with 70% ethanol. After 20 minutes of fixation, the Tcx_2 was removed from the transducer coupling, and the preparation was stored in 70% ethanol. Several days to several weeks after the experiment, the preparation was dissected and the lengths of the experimental Tcx_2 (Lref) and of the contralateral control Tcx_2 were measured with an ocular micrometer. The data presented are only from preparations in which Lref was between 95% and 105% of the length of the control muscle. The mass of experimental muscles was determined after rehydration in saline. Schistocerca flight muscles lose 18% of their fresh weight when rehydrated in saline from 70% ethanol (unpublished observation). All masses were corrected for this loss. The mean length of the control muscle was 8.1 mm (s.d.=0.29, n=17), and the mean cross sectional area, calculated as the ratio of mass and length, was 0.58 mm^2 (s.d.=0.077).

RESULTS

Passive tension and stress relaxation

When a resting muscle is stretched beyond the normal in vivo length, its resting force increases. If the stretched muscle is held at its new, longer length, there is a decay

in the observed force (Fig. 2.1). This decay in force is called stress relaxation. Stress relaxation was measured at strains ranging from 5% to 30% of the muscle length. The peak force, measured at the end of stretch, ranged from 40 mN to 125 mN (mean=78 mN) following a 10% stretch from Lref, and from 109 mN to 172 mN (mean=148 mN) following a 30% stretch. By 2 minutes after the stretch, forces had declined to about 17% of the peak values, and by 15 minutes after stretch the forces had declined to about 13% of their peak values [to 7.8 mN (s.d.=0.9 mN) following the 10% elongation; to 31.2 mN (s.d.=0.9 mN) after the 30% stretches]. In a few trials, a muscle was held stretched for 2 to 4 hours. In these trials, the force continued to decline throughout the observation period.

Both the peak force immediately following stretch and the residual force after a period of time increased as muscle strain was increased (Fig. 2.1). Responses to similar stretches were similar on sequential trials.

The time course of force decay was fit to a multiple exponential function of the form:

$$F = A_1e^{-t/K1} + A_2e^{-t/K2} + A_3e^{-t/K3}...+ A_ne^{-t/Kn}.$$

where F = force and t = time

$A_1...A_n$ = force constants

$K_1...K_n$ = time constants

The logarithm of the force was plotted against time after stretch, and each term ($A_ne^{-t/Kn}$) found by successive exponential curve 'peelings' of the straight-line por-

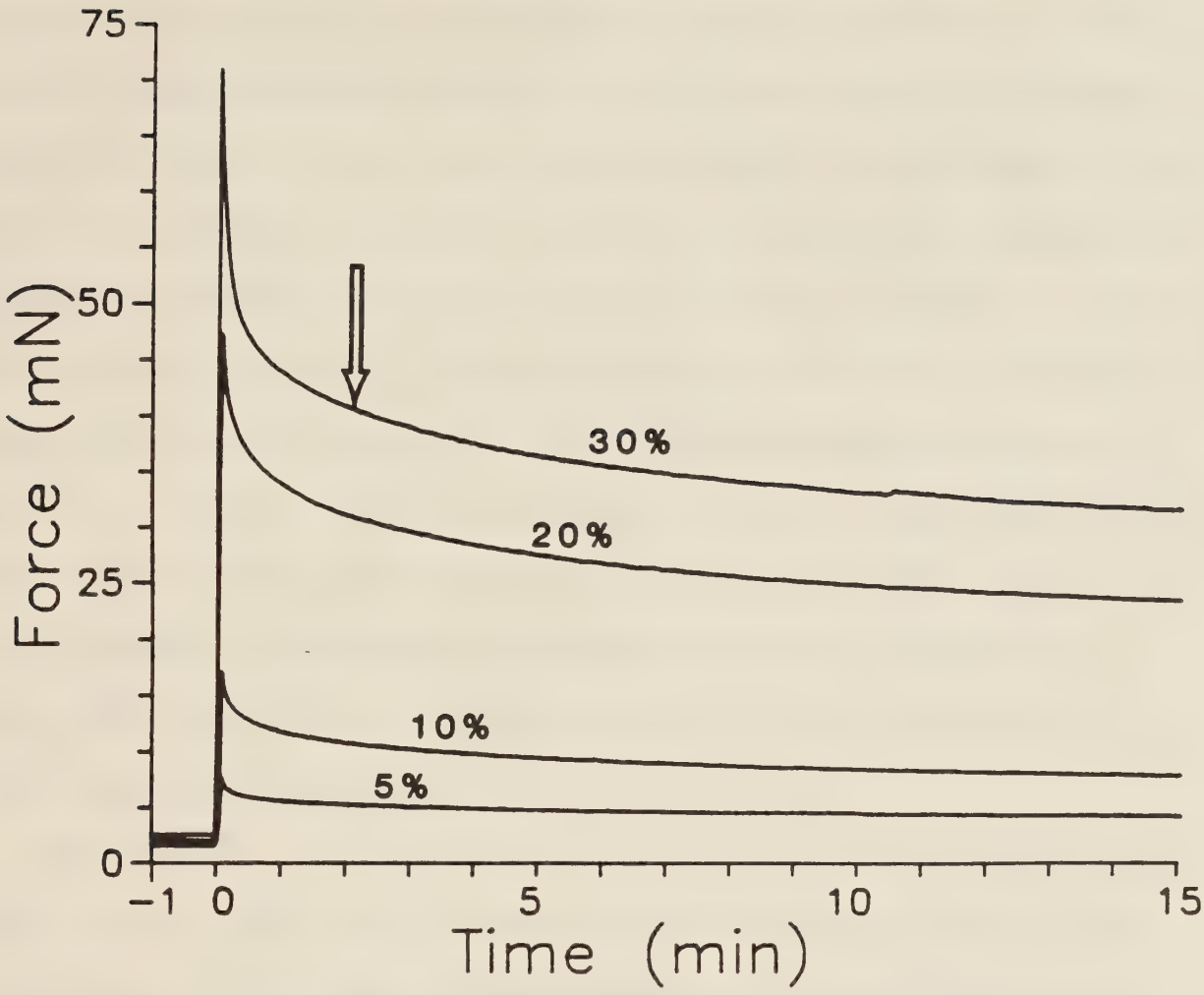

Fig. 2.1. Decline of passive muscle force following stretch. The curves are all from the same muscle, which was stretched to and held at a length above the normal in vivo length. The amplitude of stretch is indicated above each trace. The muscle was returned to normal length for more than 4 minutes between stretches.

tion of the curve at long elapsed time (see Riggs (1963) for a discussion of the technique). Some of these experimental data were also fit to multiple exponential functions by nonlinear least-squares computer routines. The several least-squares curve fitting routines utilized produced problems by the non-convergence of the program or by the establishment of local minima. Results from these routines were found to depend greatly on the estimated parameter starting values. These routines worked best when the starting values chosen were those determined with curve peeling. Use of a computer curve fitting routine may be a useful means of quantifying the goodness of fit, or of 'fine tuning' parameter estimates found by exponential curve peeling, but it was not found to be a practical first-approach procedure.

With curve peeling techniques, two or three exponential terms (Fig. 2.2A) were inadequate to describe data points over the full 15 minute time course of stress relaxation, while four time constants did give a reasonably accurate representation of the declining force (Fig. 2.2B). The time constants, K, and force constants, A, of decay were calculated (Table 2.1) for preparations held stretched for 15 minutes. In these preparations, the slowest time constant, K_1, was found to be more than an hour, with additional slow to moderate time constants of a few minutes and of tens of seconds. The fastest time constant detected by our protocol was a few seconds (in one preparation movement

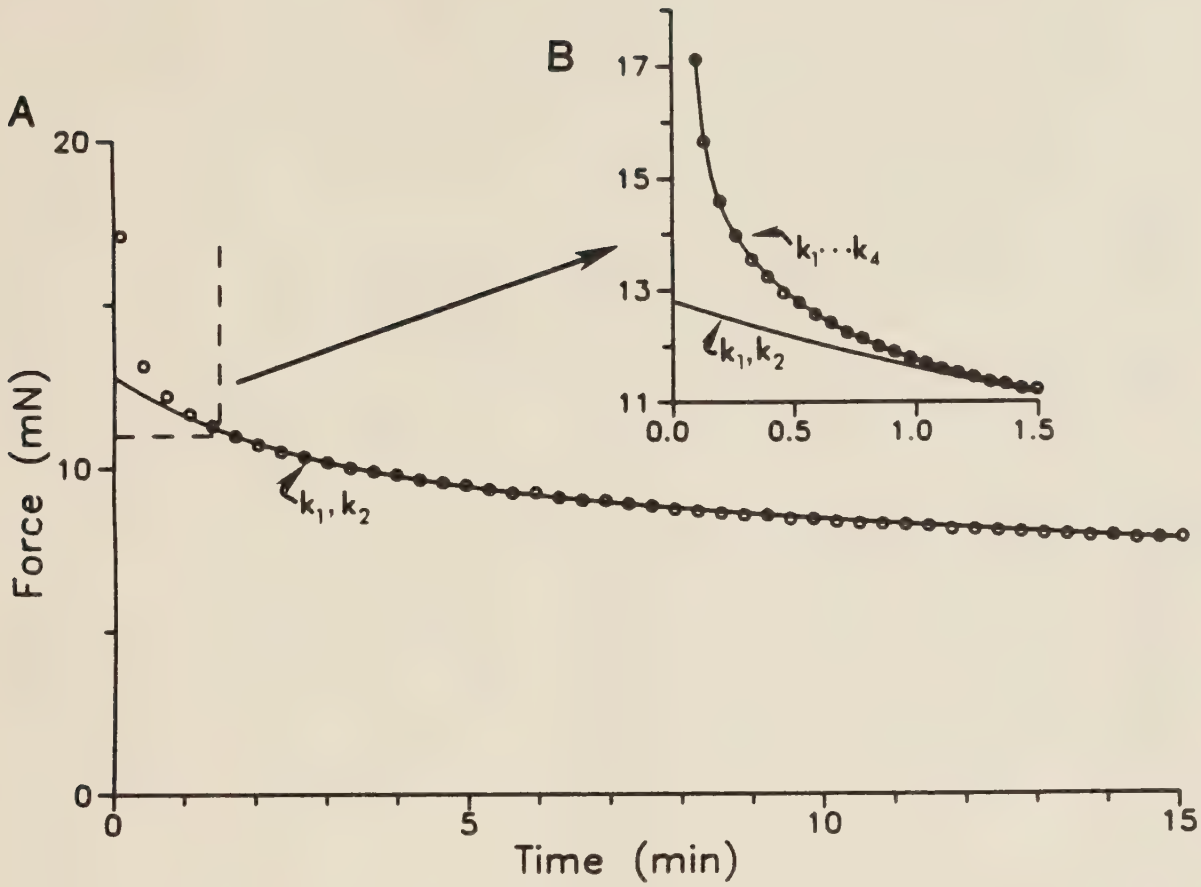

Fig. 2.2 Stress relaxation for a muscle stretched to and held at 110% of the normal <u>in</u> <u>vivo</u> length.
A. The data after one to two minutes can be fit to the sum of 2 exponential components (the solid line, $Y = A_1 * e^{-t/k1} + A_2 * e^{-t/k2}$), but this function does not fit the data points well at shorter times.
B. A function including 4 exponential components fits the data reasonably well over the entire time range examined. The time constants, $K_1 \ldots K_4$ were 69, 2.4, 0.31 and 0.04 min. These data are from the preparation shown in Fig. 2.1. For clearer presentation, only 3 pts per min are shown in A and 15 pts per min in the insert, B.

Table 2.1. Time constants (Kn) and Force constants (An) found by fitting stress relaxation data to summed exponential functions. The expression used to describe stress relaxation was:
F = A1*exp(-t/K1) + A2*exp(-t/K2) +A3*exp(-t/K3)...An*exp(-t/Kn).

A. Values from 4 preparations at 10% strain

	F1		F2		F3		F4	
	A1 mN	K1 min	A2 mN	K2 min	A3 mN	K3 min	A4 mN	K4 min
mean	8.8	113	2.5	2.3	2.9	0.32	14.0	0.08
s.d.	0.73	60	1.3	0.28	0.74	0.07	11.1	0.07
n	4		4		4		3	

B. Values from one preparation at varied strains. The stress relaxation curves for this preparation are shown in Fig. 2.1. Note that the time constants are essentially independent of the amount of strain.

	F1		F2		F3		F4	
% Strain	A1 mN	K1 min	A2 mN	K2 min	A3 mN	K3 min	A4 mN	K4 min
5	5.0	91	1.0	2.0	0.71	0.30	2.3	0.08
10	9.7	69	3.1	2.4	3.43	0.31	21.5	0.04
19	28.1	82	8.6	2.5	6.35	0.41	14.0	0.09#
29	37.3	87	9.4	2.5	6.69	0.48	11.0	0.15#

In these 2 curves, systematic errors between observed and predicted forces suggested that an even better fit would be achieved with a fifth exponential term containing an even shorter time constant.

in the early record prevented calculation of the fastest exponential component). Although there was a great deal of variation between the time constants of the preparations tested, for any particular preparation the time constants did not change substantially at strains of 5% through 30%. However, each of the force constants for a single muscle did increase with increased strain.

It is clear from the muscle performance during stress relaxation that there is no stable rest force in a stretched muscle, at least not for the first several hours following stretch. The values obtained for passive tension depend on when they are measured after the initial strain. In the following, passive force was arbitrarily defined as the force 2 minutes after the cessation of stretch. By that time, the rate of force change was 5% to 7%/min. Had longer intervals between stretch and measurement of passive force been used, the rate of force decay at the time of measurement would have been smaller, as would have been the values of passive force. Practically, there are problems of morbidity and decay with very long intervals. Two minute intervals was a reasonable compromise between the competing demands of minimization of measurement variability, collection of sufficient data points to characterize the curve, and minimization of pathological changes.

Force in the unstimulated Tcx_2 muscle at the <u>in vivo</u> length averaged only 3.4 mN [0.6 N/cm^2 (s.e.=0.1 N/cm^2, n=7)]. Passive force in stretched muscles increased

approximately exponentially with muscle length (Fig. 2.3). There was a change of slope at stretches greater than about 125% of the *in vivo* muscle length, which might have been due to damage to the muscle.

Active Force

The force exerted by a stimulated muscle is the sum of the passive force described above and the active force (sometimes called the active increment) produced by a muscle contraction. The tetanic force (Po) increased with muscle length to a maximum, and declined with increasing length thereafter (Figs. 2.4 and 2.5). That length at which Po is highest will be called the optimal length (Lo). Lo averaged slightly less (not significantly different) than the length of the muscle *in vivo*. The relationship between the active force and muscle length is a fairly broad curve, with the 50% point of the rising and falling limbs occuring at 80% Lo and 130% Lo respectively (Fig. 2.5). The active force in Fig. 2.5 is shown as a fraction of the preceding contraction at Lref. This normalization was done in order to compare the forces at different muscle lengths without interference from the effects of fatigue or injury. The curve may be artificially elevated at lengths above 125% Lo because the tetanic and twitch tension at

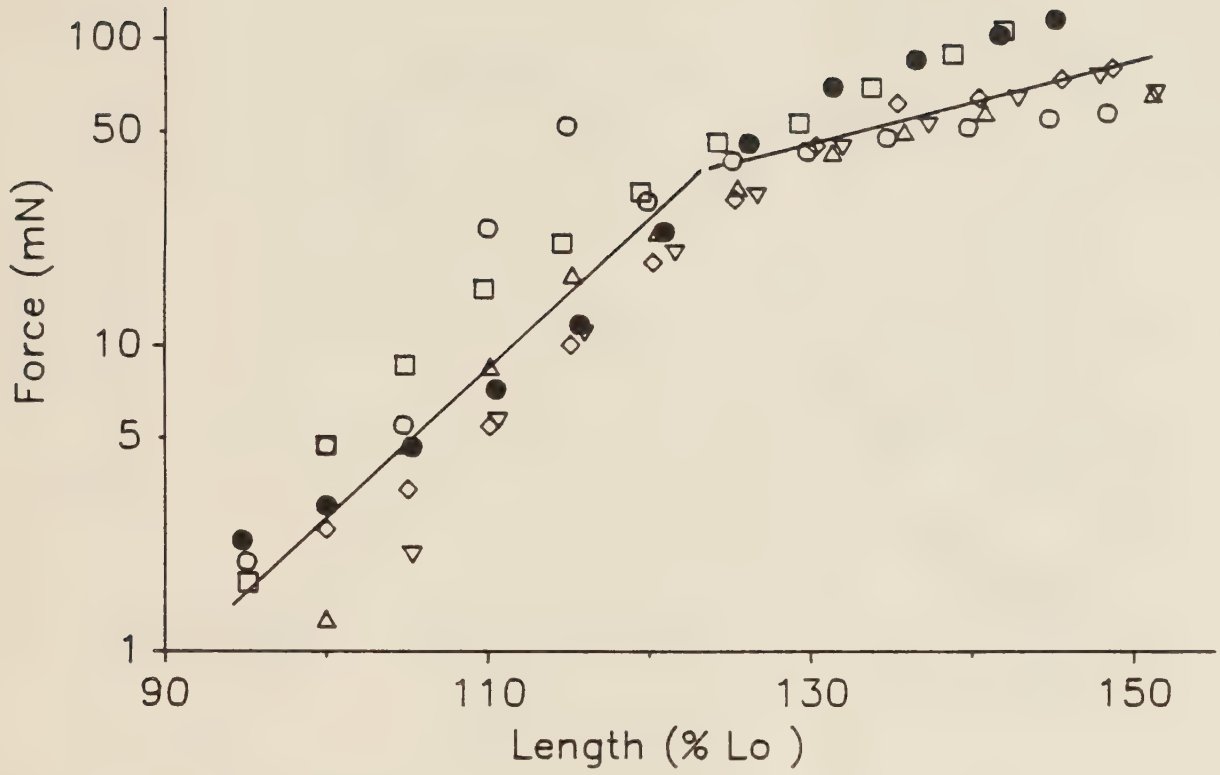

Fig 2.3. Passive force in the Tcx_2 as a function of muscle length. Lo is the muscle length at which tetanic tension is maximal. Separate straight lines have been fit to the data in the regions below 125% Lo and at greater than 125% Lo.

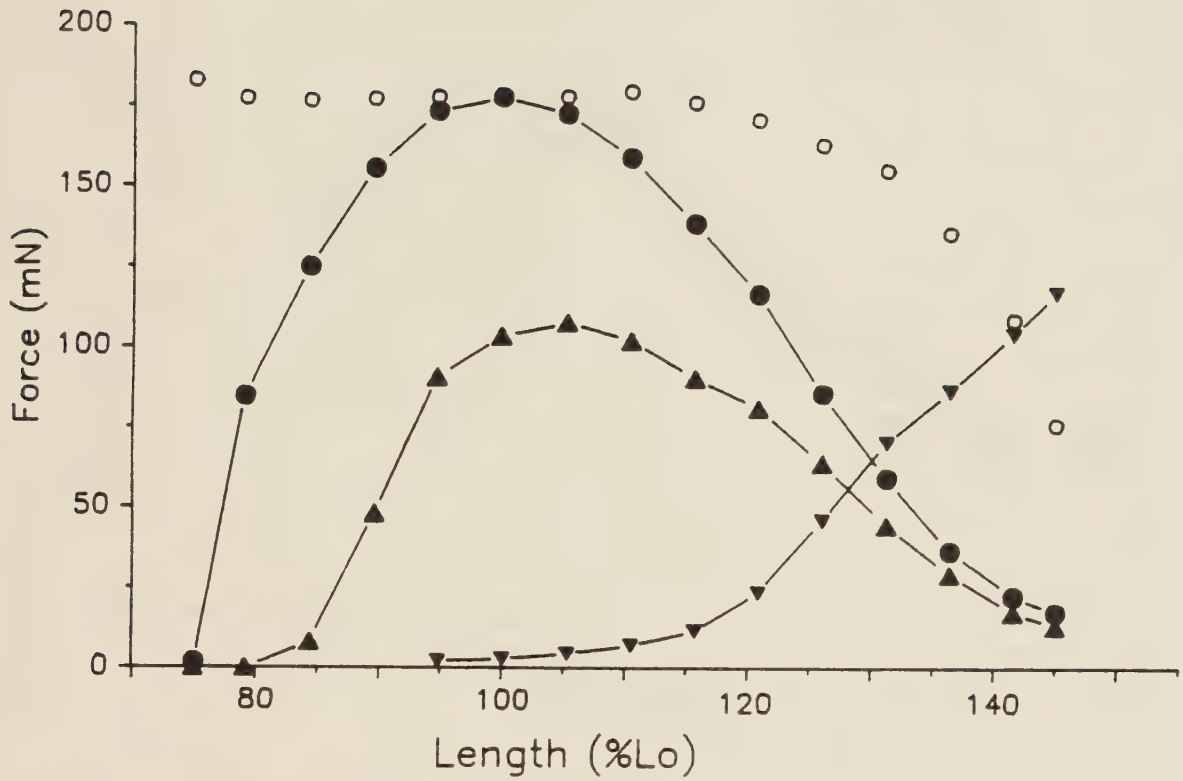

Fig. 2.4. Passive and active force in the Tcx_2 as a function of muscle length. In this preparation, Lo was 7.7 mm. Inverse triangles show the passive force at each muscle length. The active increment of force (that is, the increase in force above the passive force at that length) is shown at each experimental length (triangle=twitch, solid circle=tetanic). The open circles show the active tetanic tension in the trial at reference length preceding the trial at the corresponding experimental length. The active force at reference length dropped rapidly after stretch to, and contractions at, lengths above 130% Lo.

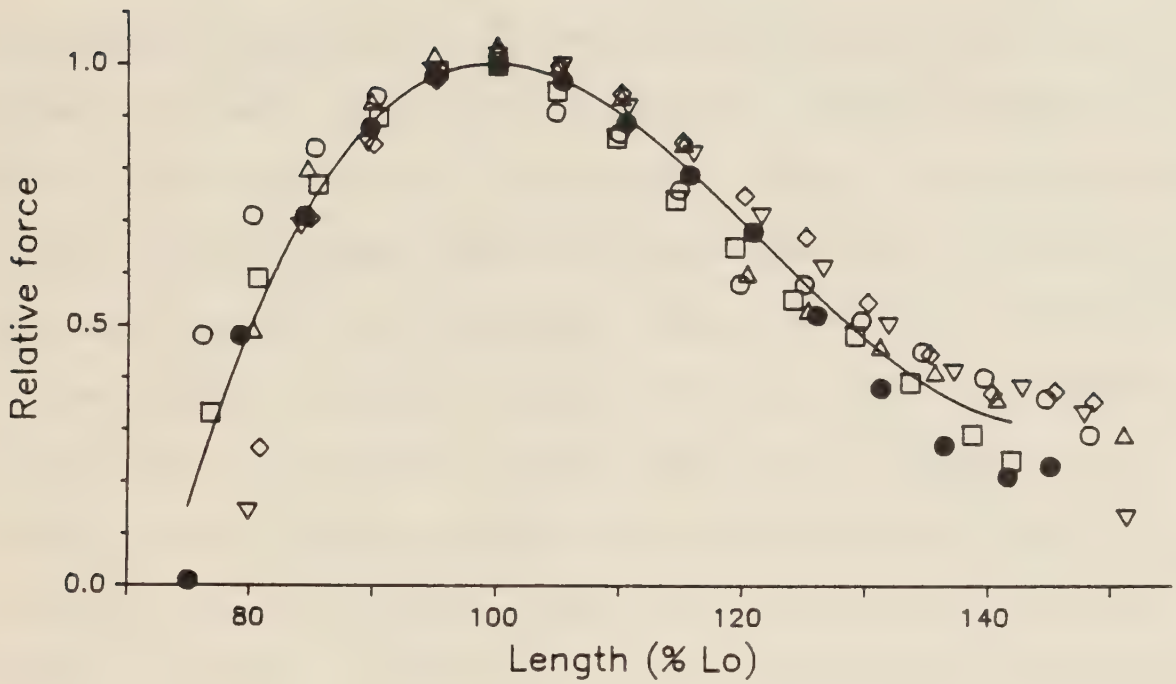

Fig 2.5. The active tetanic force in 6 preparations as a function of muscle length. Each preparation is represented by a different symbol. The solid circle is the same preparation as shown in Fig. 2.4. Force is shown as a fraction of the preceding tetanic tension (Po) at reference length. The average Po at reference length at the beginning of each experiment was 32.4 N/cm^2 (s.e.=1.7 N/cm^2, n=7). Since the reference forces were reduced after long extensions, this presentation could exaggerate the relative forces at long lengths. The solid line was fit by least squares regression to a third order polynomial.

return to reference lengths was depressed (e.g. Fig. 2.4), and the reduction of control tetanic tension increases relative forces which are based on them as a reference value. The loss of active force following long stretch may have been a result of tearing of muscle membranes (sarcolemma or sarcoplasmic reticulum), or of disrupting part of the tracheal or nerve supply to the muscle. The depression of active force was partly reversible. After 4 to 10 minutes at Lref, twitch force typically increased by 15% to 20% over that immediately following the return from stretch.

The maximum twitch tension (Ptw) occurred at a muscle length that was slightly longer than that at which the tetanic tension was maximum (mean difference=0.33 mm, s.e.=0.07, n=6; $p < .001$). The ratio of twitch to tetanic tension (Ptw/Po) in the Tcx_2 is moderately high, averaging 0.67 (s.e.=0.02, n=7) at the *in vivo* length. There was a gradual decrease in the control Ptw/Po over the course of most experiments, possibly reflecting gradual muscle fatigue. The active twitch force curve dropped more steeply at lengths below Lo than did that of tetanic force (Fig. 2.4, Table 2.2), but less rapidly as muscle length was increased above Lo. The different shapes of the twitch and tetanic curves resulted in an increase in Ptw/Po as muscle length was increased (Table 2.2).

Table 2.2. Twitch tension and twitch/tetanic force ratio in the Tcx_2 at 25 ^{o}C. Lo was 7.9 mm (s.e.=0.09 mm), twitch force at Lo was 21.7 N/cm^2 (s.e.=1.09 N/cm^2). Values are given as mean (±s.e.), n=6-7.

Muscle Length % Lo	Twitch Tension mN	Twitch/tetanic ratio
90	67.6 (±8.0)	0.42 (±0.05)
100	123.9 (±4.6)	0.67 (±0.02)
110	114.2 (±5.2)	0.69 (±0.02)
120	85.9 (±5.9)	0.74 (±0.02)

Lo = optimal muscle length

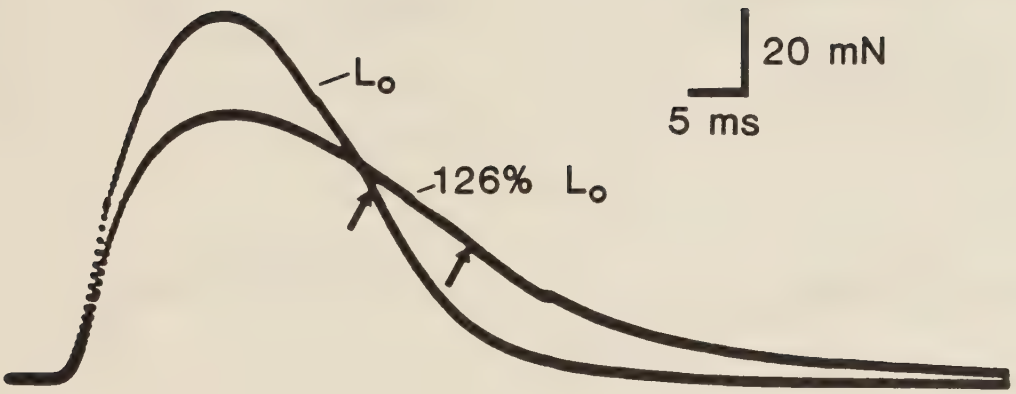

Fig. 2.6. Twitches at Lo and 126% Lo. The twitch at Lo was recorded 2 mins after that at the longer length. The resting tension of the muscle at the two lengths has been made to coincide in order to facilitate comparison of the two twitches. The arrows are at 0.5 maximum force.

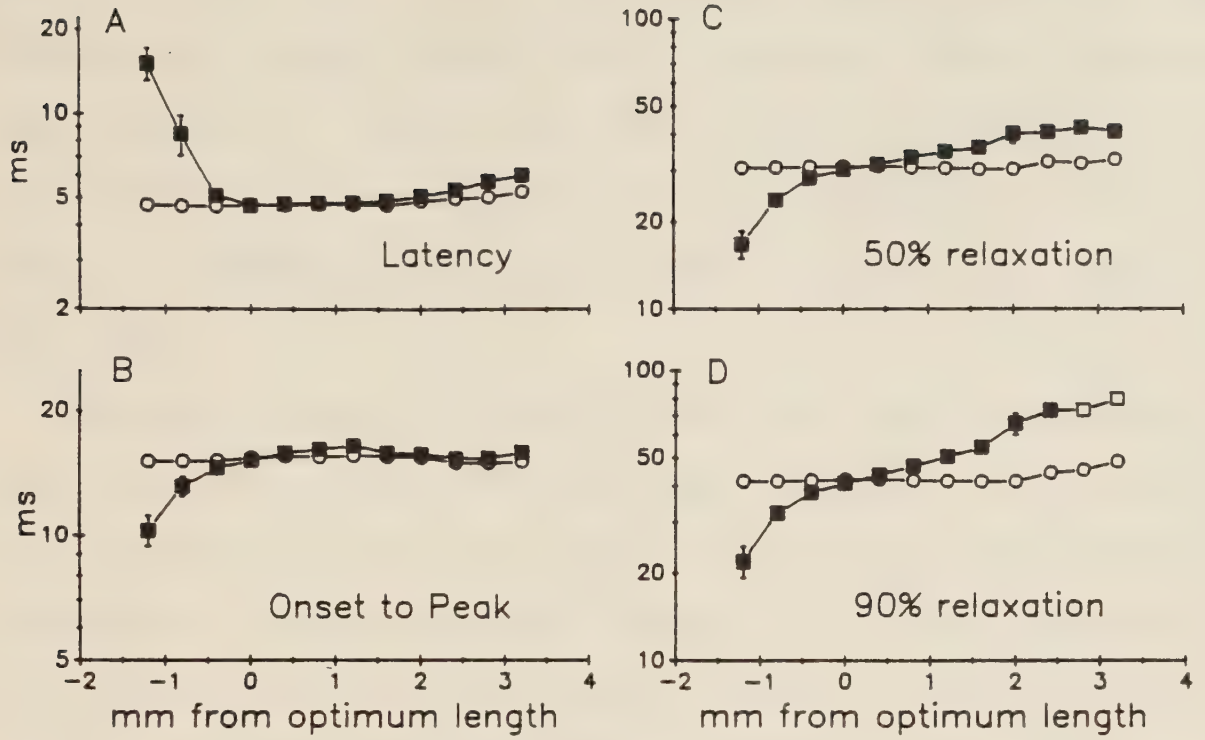

Fig. 2.7. Twitch contraction and relaxation times in the Tcx_2 as a function of muscle length (25 °C). Solid squares are average values from 6 animals at the muscle lengths shown on the abscissa. The circles are the preceding, reference length values. In two preparations at Lo+2.8 mm and one at Lo+3.2 mm the twitch duration to 0.9 relaxation was longer than the data sampling period (about 96 ms). The estimated 0.9 relaxation time was close to 98 ms, so that value was arbitrarily used for these preparations. Therefore, the mean 0.9 relaxation time at the longest lengths (open squares) are underestimates. Standard errors are shown in the cases where they are larger than the symbols. Time is plotted logarithmically so that similar relative changes in the parameters shown will result in similar displacement from the reference values.
At very short lengths, the onset of measured force is delayed until existing slack is taken up by the contracting muscle. Therefore the latency (stimulus to force onset) is overestimated and the contraction time from force onset to twitch peak is underestimated at these lengths.

Twitch time course

Both twitch amplitude and time course are functions of muscle length (Fig. 2.6). Peak twitch force was lower, and relaxation time was longer in the stretched muscle than at Lref (Fig. 2.7). There was little change in twitch rise time except at long muscle lengths, in which there was a small (not statistically significant) increase in twitch rise time.

Measured latency between stimulation and force onset depends on such things as electrode placement and stimulation intensity. While the value for latency varied among preparations, the value for latency in a single preparation at a given length was reasonably constant. The stimulus to response latency increased slightly at longer muscle lengths (Fig. 2.7).

DISCUSSION

Stress relaxation in a stretched resting muscle is initially rapid but becomes progressively slower. The equivalent, but reversed, changes can be observed after a stretched muscle is returned to rest length. The shortened muscle is slack at first, and several minutes elapse before the usual resting force is re-established. Stress relaxation in a stretched muscle, as well as in a muscle returned to rest-length after the stretch, is hastened by

muscle contractions (Ullrick, 1970). The tension decay in the stretched Tcx_2 muscle was continuous. As was noted in early mechanical studies of frog muscle (Buchthal, Kaiser & Knappeis, 1944), the passive length tension curve for a muscle depends on when, after the end of stretch, the measurements were made. The relaxation processes which became dominant late in the relaxation of the Tcx_2 had long time constants, and there was no evidence for a non-zero asymptote -- implying that the resting tension in the stretched muscle would eventually decay almost completely. Tension in a stretched, unstimulated muscle is classically described as being due to stretching a Parallel Elastic Component (see Hill, 1949; Jewell & Wilkie, 1958). Since the passive muscle tension showed visco-elastic components, with none that were strictly elastic, a better term for the Parallel Elastic Component would be the Parallel Stiffness Component.

It has not yet been determined which components bear the passive tension of a stretched muscle. Ramsey & Street (1940) suggested that the equilibrium resting tension of stretched muscle was due to the sarcolemma, and that the "muscle substance itself behaves plastically." This early view has been displaced, because of evidence suggesting that most of the resting tension resides in the myofibrils (Buchthal & Weis-Fogh, 1956; Podolsky, 1964; Ullrick, 1970; Magid & Law, 1985). However, the source of the stiffness in the myofibril has yet to be firmly identified. A very

thin filament was early seen connecting the A filament to the Z line in insect fibrillar flight muscle (Auber & Couteaux, 1963 cited by White, 1983) and thought to be the basis for the high resting tension of that muscle (White, 1983). Connecting filaments have now also been seen in vertebrate skeletal muscle (Magid et al. 1984). It has been proposed that C-filaments, containing the very large proteins nebulin and titin (Horowits, Kempner, Bisher, & Podolsky, 1986), are responsible for much of the passive tension, as well as for keeping the thick filament centered in each sarcomere.

Stress relaxation in vertebrate and arthropod skeletal muscle has been described as being a time dependent process, fit by a fast and a slow exponential term (Hoyle, 1983; Chapple, 1983, 1987; Magid & Law, 1985). In the locust Tcx_2, on the other hand, more than two time dependent terms were required to fit the data points. The difficulty of adequately fitting data with multiple exponential terms either by curve peeling or by nonlinear least squares regression analysis is well known (Riggs, 1963; Landaw & DiStefano, 1984) but it is clear that 2 exponential terms, found either by exponential curve peeling or by nonlinear regression analysis, failed to fit these data adequately (see Fig. 2.2). At least 4 exponential terms are needed to fit stress relaxation over a 15 minute period, and it is possible that more terms would be needed to fit force decay over longer periods.

Many biomaterials such as collagen, and even crystalline polymers such as polyethylene, show multiple component relaxation spectra rather than simple one or two exponential relaxation curves (Wainwright, Biggs, Currey, & Gosline, 1976). It is proposed that the different domains of these large molecules relax at different rates, which leads to multiple component relaxation spectra. The relaxation curve formed by multiple spectra will, under some circumstances, be fit by a power function ($F=At^{-k}$). A power function is linear when both force and time are plotted on logarithmic axes. A power function was used to describe the stress relaxation of an insect fibrillar flight muscle (White, 1983). A power function did not, however, fit stress relaxation in the Tcx_2. When plotted logarithmically, the data diverged from a straight line at both short (less than about 30 s) and long (greater than 2 to 3 hours) times.

The maximum active tension in the Tcx_2 is about 32 N/cm2 which is somewhat greater than most values reported for frog muscle [e.g., for frog sartorius: 20 N/cm^2 at 2 ^oC (Jewell & Wilkie, 1958); 33 N/cm^2 at 20 ^oC (Close, 1972); 24 N/cm^2 at 25 ^oC (Renaud & Stevens, 1981); 27 N/cm^2 at 25 ^oC (Rome, 1983)]. The relationship between active force and muscle length (Figs. 2.4 and 2.5) was not as broad as for some frog muscles, but the active force curve was less sharply peaked than that reported for the locust DLM (Weis-Fogh, 1956). The muscle force was 0.5 Po or more over a

range equal to 50% of the optimal muscle length in the Tcx_2. The comparable range in the DLM is 40% of the optimal muscle length (Weis-Fogh, 1956a). Whole frog sartorius develops 0.5 Po or more over 53% to 62% of its optimal muscle length (Wilkie, 1956; Aubert, Roquet, & Van der Elst, 1951).

The twitch tension was maximum at a muscle length that was slightly longer than the in vivo length. The length tension curve for twitches was somewhat narrower than that for the tetanic contractions, but the whole length-tension curve for twitches was shifted to the right, so that at long muscle lengths the maximum twitch force more closely approached the tetanic force (Fig. 2.4). A pronounced increase in muscle relaxation time (Figs. 2.6 and 2.7) occurred at long muscle lengths, and was evident in both the twitch and tetanic contractions. Similar increases in twitch relaxation times have been reported for the frog sartorius (Close, 1972) and a katydid wing muscle (Josephson, 1973).

An increase in the duration of the active state would increase the Ptw/Po in a muscle, and could also increase the relaxation time. The longer active period would allow more time for force development -- hence the larger Ptw. An increase in the duration of the active state would not, of itself, affect the tetanic tension, since the muscle is already fully active at the plateau of tetanic force development. A direct correlation was found between muscle

length and the duration of the active state in the frog sartorius (Ritchie, 1954). However, if the active state were to become more prolonged, the time to peak tension would be expected to lengthen, which did not happen in the locust Tcx_2 [nor in the katydid wing muscle (Josephson, 1973)].

The lengthened period of relaxation and the higher twitch to tetanus ratio in the Tcx_2 can best be explained by an increased calcium sensitivity in the muscle. It has long been known that a stretched mammalian heart beats more strongly (the Frank-Starling mechanism). Myofilament calcium sensitivity has now been shown to be increased by stretch in cardiac muscle as well as in a number of skeletal muscles (Ruegg, 1987; Stephenson & Wendt, 1984), and may be modulated by troponin C (Babu, Sonnenblick, & Gulati, 1988). If the muscle were more sensitive to calcium, the force at equivalent calcium concentrations would be higher and the muscle would develop more force and relax more slowly even though there were no changes in the liberation and sequestration of calcium by the sarcoplasmic reticulum. The increase in calcium sensitivity at long lengths is much greater in partially activated skinned muscle fibers than in fully activated fibers (Stephenson & Wendt, 1984), which correlates well with the increased twitch to tetanus ratio and the longer muscle length of the maximal twitch tension than of the maximal tetanic tension that was seen in the Tcx_2.

Recently considerable attention has been given to high frequency stiffness of muscles (e.g., Farrow, Rossmanith, & Unsworth, 1988; Tsuchiya & Sugi, 1988); for comparisons of high frequency stiffness and static tension see Haugen & Sten-Knudsen, 1981, and Schoenberg & Wells, 1984). Since high frequency muscle stiffness was not measured for the locust Tcx_2, it is not known if the Tcx_2 is similar or dissimilar to vertebrate striated muscle in the high frequency domain. However, the static resting tension of the locust Tcx_2 is less than that reported for the locust DLM and is not dissimilar to that found in whole frog muscle (Fig. 2.8B). It is thus incorrect to describe insect muscles as being unusually stiff and inextensible. Since the length-tension relationships for both active tension (Fig. 2.5) and passive tension (Fig. 2.8B) in the Tcx_2 are similar to that of a typical vertebrate skeletal muscle, it is hardly surprising that the total tension relationships (Fig. 2.8A) also are not substantially different in the frog leg muscle and the insect wing muscle.

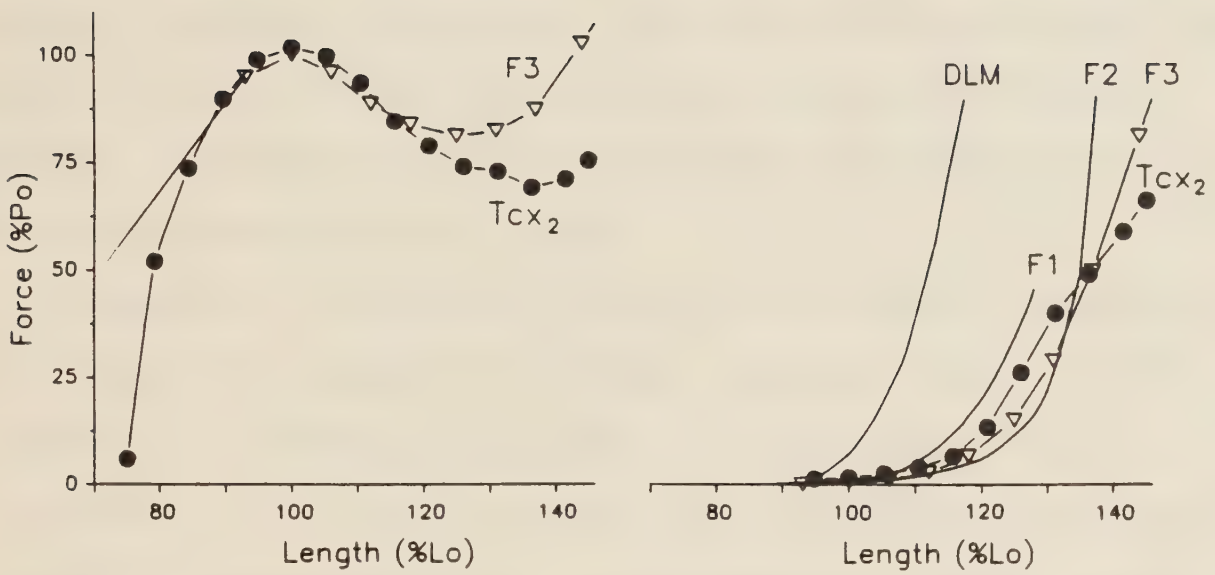

Fig. 2.8. Active and passive force as a function of muscle length
A. Total tension during a tetanic contraction in a locust wing muscle (Tcx_2) and a frog sartorius muscle (F3). The frog values are from Aubert et al. (1951).
B. Passive tension in locust wing muscles and frog leg muscles. DLM = locust dorsal longitudinal muscle (after Weis-Fogh, 1956a); F1, F2, F3 = frog sartorius muscles (F1 from Wilkie, 1956; F2 from Hill, 1970; F3 from Aubert et al. 1951).

SUMMARY

Tension in a resting muscle increases when the muscle is stretched. If the stretch is maintained, the tension decays (stress relaxation). In the metathoracic second tergocoxal muscle (Tcx_2) of the locust, Schistocerca americana, a multi-exponential function with 4 or more time constants was required to adequately describe the time course of stress relaxation. The slowest time constant was over 60 minutes. The time constants were independent of strain. Tension continued to diminish even when stretch was maintained for 2 to 4 hours.

Tension in a stretched, unstimulated muscle increased with increased length of stretch. Resting tension in the locust Tcx_2 at the in vivo length is estimated to be 1 N/cm^2 or less. At 10% strain, resting tension was about 2 N/cm^2. The stiffness of the unstimulated locust muscle was similar to that of unstimulated frog muscles.

The active tetanic tension was maximum (average = 32.4 N/cm2) at slightly less than the in vivo muscle length. Tetanic tension was 50% or more of its maximal value over a range of 80% to 130% of the in vivo muscle length.

The active twitch tension was maximum at slightly greater than the in vivo muscle length. The twitch/tetanic force ratio increased with muscle length.

Twitch relaxation time increased with muscle length, but time to peak twitch force was nearly independent of muscle length in a stretched muscle.

CHAPTER 3

FORCE VELOCITY RELATIONSHIPS AT DIFFERENT TIMES DURING TWITCH CONTRACTIONS

INTRODUCTION

Muscles that are used in locomotion or other cyclic activities are active during only a part of the movement cycle. Further, they are not equally active throughout their contraction period. There is in each cycle a time when the muscle develops force, and a later time when the force wanes during relaxation. In order to understand the work capability of a muscle, it is necessary to know the force production of which the muscle is capable throughout the movement cycle, and during the transition between resting and active states.

A relaxed skeletal muscle is flaccid and has a low metabolic rate. An active muscle is stiff, capable of resisting extension and of shortening against a load, and has a high metabolic rate. The transition of muscle from relaxed to active has long fascinated naturalists and physiologists, but has proven difficult to characterize in detail. A.V. Hill, during a long career investigating the 'fundamental mechanical response' of a stimulated muscle, quantified the relationship between muscle force and velocity (Hill, 1938), and introduced the term 'active state'. In early studies the term active state was used to differ-

entiate an active muscle, with high heat production and the capacity to shorten, from a resting muscle with a low rate of heat production. Hill later modified the concept and used the term as a quantitative measure of the force generating capability of the contractile component of the muscle (Hill, 1949). This redefinition was followed by a period when many ingenious experiments were done attempting to chart the time course of the active state (see Hill, 1965 and references therein; Ritchie, 1954; Edman, 1970). Many of these studies utilized quick stretch or quick releases of the muscle. However, the time course of muscle activity is affected by muscle length (Ritchie, 1954), changes in length (Edman & Kiessling, 1971) and the stimulation history of the muscle. Most methods used to determine the intensity of the active state may, in fact, affect its intensity and duration (See Pringle, 1960 for a detailed and critical review of the active state concept).

The concept of active state, in Hill's 1949 terms, has remained controversial, and active state <u>per se</u> is now infrequently investigated. It is, however, obvious that the ability of the muscle to develop force and to shorten does change with time after stimulation. I have characterized the changing force-velocity relationships during a twitch in order to monitor these changes in mechanical capacity. The term 'state of activation' (SA) is used in this chapter to indicate a quantitative measure of the capacity of a muscle to shorten and to do work. Hoyle

(1983) used the term 'state of activation' as a measure of "the maximum tension that can just be borne by a muscle at a given instant." I extend this definition to include the force generating capacity of a muscle during shortening. A measure of the state of activation should provide a background for assessment of the potential work and mechanical power output of a muscle during normal movements. This characterization should also be useful to the study of the molecular events during muscle contraction, particularly those events during the onset and decay of activity.

MATERIALS AND METHODS

The metathoracic second tergocoxal muscle (Tcx_2) of adult, male locusts, Schistocerca americana, was used in all experiments. Animals were obtained from a laboratory culture, maintained at 29 ^{o}C and on a 16 hour light:8 hour dark cycle. Animals were fed growing wheat seedlings and a commercial dog food based on soy and beef.

The Tcx_2 is an indirect wing levator and a coxal remoter. This muscle is approximately 8 mm long and weighs about 4 mg. All experiments were done on in vivo muscle preparations at 25 ^{o}C. 25 ^{o}C is lower than the thoracic temperature during sustained flight (30 ^{o}C), but animals with body temperatures of 25 ^{o}C are capable of initiating and continuing flight (Weis-Fogh, 1956b). The lower temperature was selected because operating at the cooler tem-

perature slows the muscle and makes less demanding the problems of force clamp and velocity measurements.

The muscle was prepared for mechanical measurements with a minimum of dissection. Locust flight muscle is very aerobically dependent (Weis-Fogh, 1956b); therefore, care was taken during dissections to keep intact the tracheae that bring oxygen to the Tcx_2 muscle. The metathoracic ganglion was exposed and removed, thus destroying normal neural input to the muscle. The coxa was isolated of all its attachments except that of the Tcx_2 apodeme, and the coxal cuticle was trimmed down to a small piece surrounding the apodeme. The muscle was later attached to an ergometer by slipping this piece of coxal cuticle into a hook attached to the ergometer. Electrodes (50 um silver wires) were implanted in the dorsal insertion of the muscle, and the animal was fastened on its back to a platform with epoxy glue. During measurements the muscle was occasionally moistened with locust saline (Usherwood & Grundfest, 1965). The thoracic temperature was monitored by a thermistor inserted in the thorax through the contralateral coxa. The thoracic temperature was maintained at 25 ^{o}C by adjusting the intensity of a microscope lamp illuminating the thorax.

Locust wing muscles function in twitch or twitch-like contractions during flight, activated by a single action potential with occasional short bursts of 2 or 3 action potentials (Wilson & Weis-Fogh, 1962). The Tcx_2 is quite

homogeneous, having only 3 motor units (Kutsch & Usherwood, 1970), all of approximately the same time course.

Stimuli for muscle contractions were 0.5 ms shocks through the implanted silver wire electrodes. During measurements of twitch contractions, one shock was delivered at 1.3 times the minimum voltage needed to activate all three motor units. Tetanic stimuli were 1.5 times the threshold intensity. Tetani were induced with multiple stimuli at a frequency of 250 Hz. Tetanizing stimulus bursts lasted 65 ms. In order to keep the muscle in a steady state, twitches were produced repeatedly at 2 per minute or tetani at one per minute throughout any series. The Tcx_2 twitch is potentiated immediately following a tetanus. To minimize the effects of post-tetanic potentiation, all measured twitches were at least 10 minutes after any tetanic stimulation.

Muscle force and length were measured and muscle length was controlled with an ergometer. The ergometer consisted of a strain gauge mounted on a shaft attached to a puller motor (Ling Model 102A Shaker). The strain gauge was contructed from a pair of semi-conductor elements (Pixie Model 8121A, Endevco Corp.). An insect pin, bent into a hook at the distal end, was fixed to the strain gauge as an attachment point for the muscle. The resonant frequency of the transducer and attached pin was about 3 KHz. The shaft from the puller passed through a teflon bearing which limited shaft movement to the axis of the

puller motor. A pair of vanes attached on opposite sides of the shaft partially interrupted light paths between light emitting diodes and phototransistors. The voltage output from the phototransistors was used to monitor the linear position of the shaft of the puller. The position of the shaft was under servo control. Two control modes were available, position clamp and force clamp. Dynamic stops in the servo circuitry prevented the shaft from causing the muscle to shorten or lengthen beyond preset points. In the position clamp mode the error signal for the servo loop was the difference between a control signal and the signal from the position detector on the shaft. In the force clamp mode the error signal was the difference between a control signal and the signal from the force transducer. The servo control used proportional, integral and damping feedback (PID control). The amplifications of the proportional, integral and damping feedback were individually adjustable for optimal performance.

Force and position data were collected with an A/D converter (Metrabyte Dash 16) interfaced with a microcomputer. Force and position signals were sampled at 6-12 KHz for immediate display, and were stored as computer files for later analysis. A low pass filter (2 KHz) was used in both force and position data collection.

Most experiments to be described involved a quick release from position clamp to force clamped at a preset load. The transition from position clamp to force clamp

occurred after a controlled delay following the onset of muscle stimulation. Reasonable force clamp was established within 3-4 ms from the onset of the transition. During tetanic muscle contraction, and with optimal adjustment of the servo feedback, the muscle load could be held constant, varying by less than 1% of the maximum tetanic force, for 20 ms or more (see Fig. 3.1A). Force clamp was difficult to achieve late in twitches as activity rapidly declined, and reasonably constant loads could not be maintained for more than a few ms late in twitches.

The muscle shortening velocity went through a series of transient changes following the transition from length clamp to force clamp. In part these transitions are due to early instabilities in the force clamp servo system, in part to rapid shortening of partially-unloaded series elastic elements, and in part to velocity transients inherent to the contractile machinery itself similar to those which have been described in frog muscle fibers (e.g., Podolsky, 1960). Position change and force were measured as soon as possible after the shortening velocity became reasonably constant; approximately 5 ms after the onset of the load imposition in twitches and 9-10 ms after load clamp in tetanic responses (bars, Fig. 3.1). After the initial transients, shortening velocities during tetanic responses remained quite constant for many ms. During twitches, in contrast, shortening velocities often progressively decreased with time (Fig. 3.1B). For releases that came

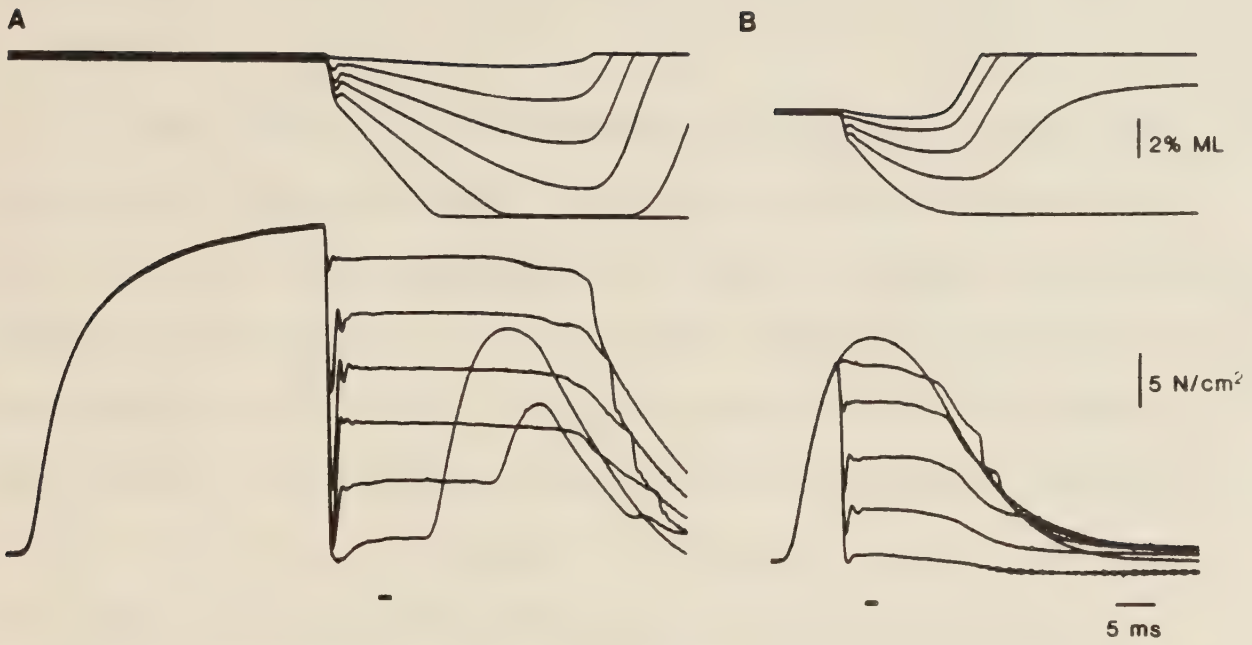

Fig. 3.1. Load clamps in twitch and tetanic contractions of a muscle.
A. Releases from isometric to isotonic contractions during the plateau of a tetanus.
B. Releases late in the rising phase of a twitch.
The traces in the top sets are position (shortening downwards), and those in the bottom sets are the corresponding forces. An isometric twitch force trace is included as a reference. During the twitch contraction, the muscle length (ML) before release to the isotonic contraction was the *in vivo* length, 8.1 mm. The initial muscle length for the tetanic contractions was slightly longer than the *in vivo* length, as indicated by the starting positions of the length traces in A and B. The thickened position baselines preceding release reflect slight changes in starting position between trials, due to differences in optimal ergometer feedback settings. Short dark bars indicate the time over which force and velocity were measured.

early in a twitch, the shortening velocity was determined from the change in muscle length over a 2 ms time interval. With releases that came late in a twitch the shortening velocity often changed rather rapidly with time, and the velocity was determined from the change in length over a 1 ms period.

At the start of each experiment the Tcx_2 muscle was attached to the ergometer, which was then positioned so that the muscle was approximately at the in vivo length, as judged by the position of the coxal remnant with respect to the surrounding cuticle. During some of the load clamps with tetanic stimulation, the muscle length was initially set at 0.2 mm (approximately 2.5% muscle length) greater than the in vivo length. The quick elastic decrease in length associated with the reduction of force from an isometric tetanic contraction to a low load can be as much as 0.25 mm. By starting with the muscle slightly longer than its usual length, it was possible to measure the shortening velocity at or near the in vivo length.

RESULTS

Muscle force and shortening velocity

The relationships between muscle force and shortening velocity were determined for muscles contracting isotonically following release from position clamp to force clamp (Fig. 3.1). Measurements were made during tetanic contrac-

tions and during twitches. With tetanic contractions the release from position clamp to force clamp occurred 50 ms after the onset of stimulation, which was within the plateau of the tetanic contraction. With twitches, releases were timed so that measurements could be made at 4 equally spaced intervals through the twitch, with the first measurement half-way through the twitch rise time (RT) as measured from tension onset to tension peak during isometric contractions. The next series of measurements was made at the time corresponding to the twitch peak; the next two at progressively longer intervals during the twitch relaxation phase. These 4 measurement times will be identified as 0.5 RT, RT, 1.5 RT and 2 RT respectively. Two RT is approximately half-way through the relaxation phase of the twitch. In order that results due to low activity would not be accentuated by fatigue, the tetanic series was the last set, immediately preceded by the series at 0.5 RT.

The tetanic force-velocity relationship for the Tcx_2 is quite consistent from preparation to preparation and is generally similar to that reported for other muscles (Fig. 3.2; see also Fig. 8 in Malamud *et al*. 1988). As was demonstrated by Hill (1938) for frog muscle and subsequently by others for a host of muscles, the relationship between force and shortening velocity is approximately hyperbolic. The rectangular hyperbola usually used to fit force-velocity data is that of Hill (1938):

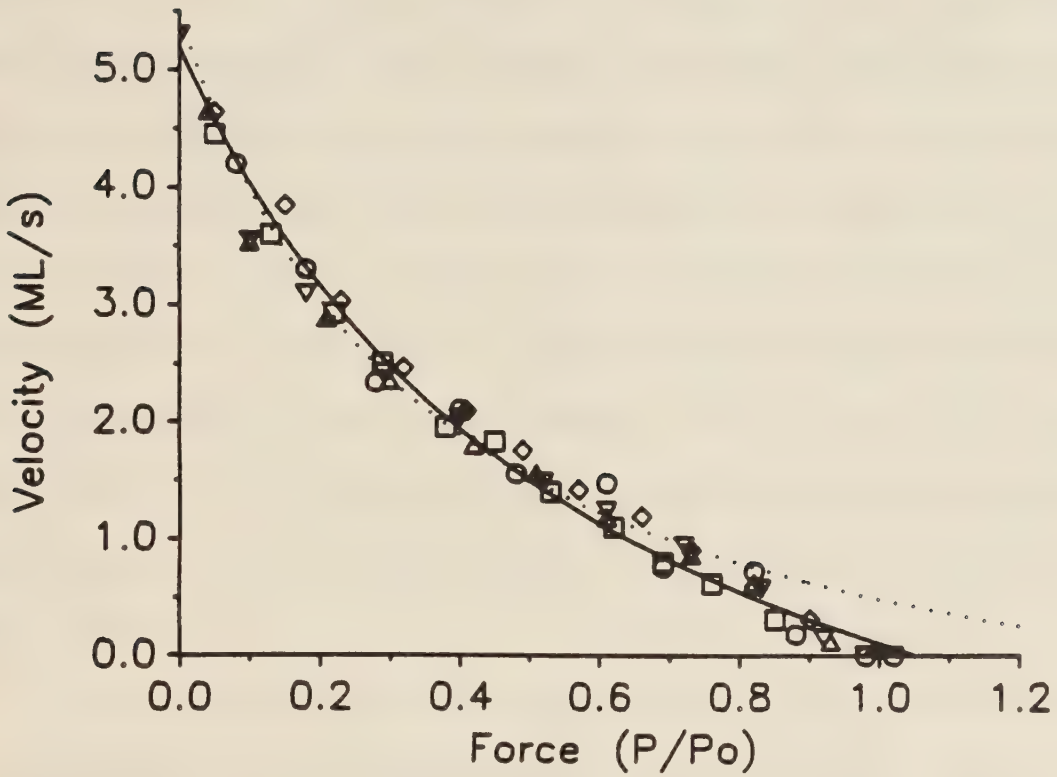

Fig. 3.2. Shortening velocity (muscle lengths/s) as a function of load during tetanic contraction. Values were obtained from release to isotonic contraction during the plateau of an isometric tetanus, as illustrated in Fig. 3.1A. The data are from 5 preparations, each of which is indicated by a separate symbol. Force values were expressed as a fraction of the maximum isometric force (Po) measured from each preparation. The curves are hyperbolae, fit using the method of Edman (1988) to the combined data set. The solid curve is the best-fit hyperbola for all data points, the dotted curve is for points excluding those with force values greater than 0.78 Po.

$(P+a)(V+b) = (P^*o+a)b$

P = muscle stress

V = shortening velocity

a, b, P^*o = constants for a given hyperbola

It has been noted that with fibers from frog muscles, the velocities at high forces seem to lie on a different curve than those at low forces, and that the fit of data points to a hyperbola was improved if velocities at high loads were deleted from the calculation (Edman et al. 1976; Edman, 1988). This was also the case with this insect muscle: the sums of squares error between measured and predicted values was somewhat reduced when the hyperbola used to fit the data points was constructed using only those points which were 80% or less of the maximum force (Fig. 3.2).

The maximum force that an active muscle can bear without lengthening is determined in several ways, and it is useful to identify and distinguish between them. Po is used to indicate maximum isometric force measured from a tetanically stimulated muscle. P^*o is used to indicate the zero velocity intercept of a hyperbola fitted to force-velocity data. For tetanic contractions P^*o is generally greater than Po. The maximum force that a muscle can bear without lengthening can be determined during twitch contractions, or during the rising or falling portions of tetanic contractions, by determining the force in a load clamp at which the shortening velocity is 0 (Fig. 3.3).

This force is equivalent to that which Hill termed the active state force (Hill, 1949) and is designated P_A. During the tetanic plateau P_A equals Po.

The maximum velocity of shortening (Vmax) calculated from the Hill hyperbola fit to the combined force-velocity data from the 5 muscles of Fig. 3.2 was 5.2 muscle lengths (ML)/s (r = 0.805). The value of the intercept of the Hill hyperbola with the zero velocity axis (P^*o) was 1.05 Po. The average Po for these muscles (measured during the plateau of the tetanic contraction) was 36.3 N/cm^2 (s.d.=1.4, n=5). The curvature of the Hill hyperbola is conventionally measured as the ratio a/P^*o. For these combined data the curvature was 0.58. Values determined for the combined data set truncated at 0.8 Po (r = 0.812) were: Vmax = 5.5 ML/s; P^*o = 1.5 Po; a/P^*o = 0.24.

During tetanic contractions the muscle is stimulated repeatedly to keep it fully activated, and the muscle shortening velocity at a given force is reasonably constant over a long period (Fig. 3.1A). With twitches, in contrast, muscle activation rises and then falls with time, and this changing state of activation is reflected in a changing shortening velocity at a given load (Figs. 3.1B, 3.3). It is because of this additional complexity with twitches that tetanic contractions are the more often studied.

In tetanic contractions and late in twitches, full force-shortening-velocity curves may be obtained by releas-

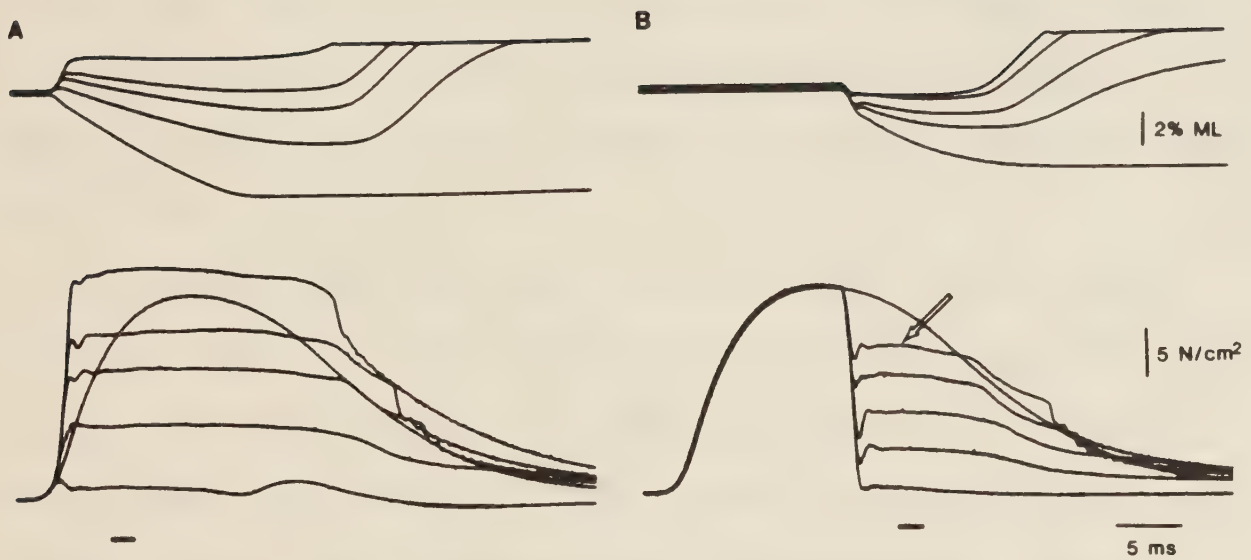

Fig. 3.3. Isotonic contractions early (A) and late (B) in the twitch. In each set of force traces, an isometric twitch is included as a reference. The arrow in B marks the force that was just adequate to prevent muscle shortening or lengthening. This force, P_A, is the 'active state force' at that time, as defined by Hill (1949). Short dark bars underneath the force traces indicate the time over which force and velocity were measured.

ing the muscle and allowing it to shorten. Early in a twitch, when the tension is still rising, it is necessary to stretch the muscle so as to increase its tension appreciably above the isometric level in order to obtain slow to moderate shortening velocities (Fig. 3.3A). As the twitch progresses, the maximum force that the muscle can bear without lengthening (P_A) decreases, as does the velocity at forces less than P_A. Late in the twitch, the muscle does not shorten at all (after the quick elastic adjustment to load) at forces well below those from which it was released (Fig. 3.3B).

The force-velocity data for twitch and tetanic contractions from the experiment illustrated in Figs. 3.1 and 3.3 are plotted in Fig. 3.4. There is little difference between the force-velocity relationship measured early in the twitch and that in the tetanic contraction. With increasing time in the twitch there is progressive decline of the velocity at any force level (Fig. 3.4). P_A fell approximately linearly throughout the twitch.

It is clear that during the course of a twitch there is a progressive shift of the force-velocity curves along the force axis. It is less obvious whether a change in the shape of the curves is associated with this shift. Often forces are expressed as a fraction of Po in plotting force-velocity data, as in Fig. 3.2. When several sets of force-velocity data are plotted simultaneously, normalizing the data to Po forces all sets to converge on Po but often

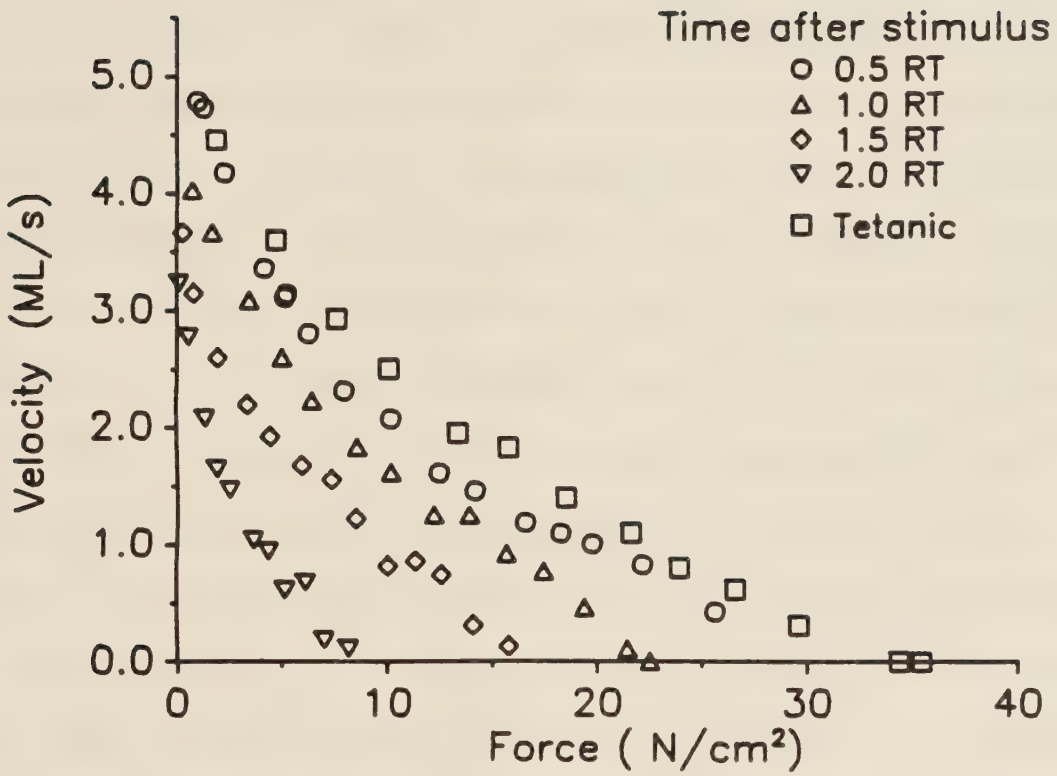

Fig. 3.4. Shortening velocity as a function of muscle load during tetanic contraction and at different times during a twitch. Releases to isotonic shortening were timed so that velocity transients were over and velocity measurements could be made at equally spaced time intervals throughout a twitch. 0.5 RT = 11.6 ms, RT = 18.1 ms, 1.5 RT = 24.6 ms, 2.0 RT = 31.1 ms.

leads to increasing dispersion of points at decreasing forces. In order to compare the shapes of the force-velocity curves during a twitch, and especially to compare points at low forces, I chose to normalize the force data to the middle of the curve. For these preparations the velocity at 0.5 Po during a tetanus averaged 1.6 ML/s (s.d.=0.06, n=5). In Fig. 3.5 the force values for each set of data were divided by the force measured or estimated at a shortening velocity of 1.6 ML/s for that set. This procedure forces all the curves to pass through a common point in the middle of the force range. The normalized positions of the points obtained early in the twitch and those from the peak of the twitch are essentially the same as the positions of points collected during the tetanic plateau. However there appears to be an increasing deviation in curve shape in successive times during the relaxation phase. Consequently, during the course of a twitch there is a progressive reduction in the maximum shortening velocity and an increase in the curvature of the hyperbola fit to the data points (Table 3.1).

Changing state of activation during a twitch

In the preceding section it was shown that through much of a twitch, the curves relating muscle force and shortening velocity are similar to those during the plateau of a tetanic contraction, but the curves for twitches become progressively displaced back toward the origin of

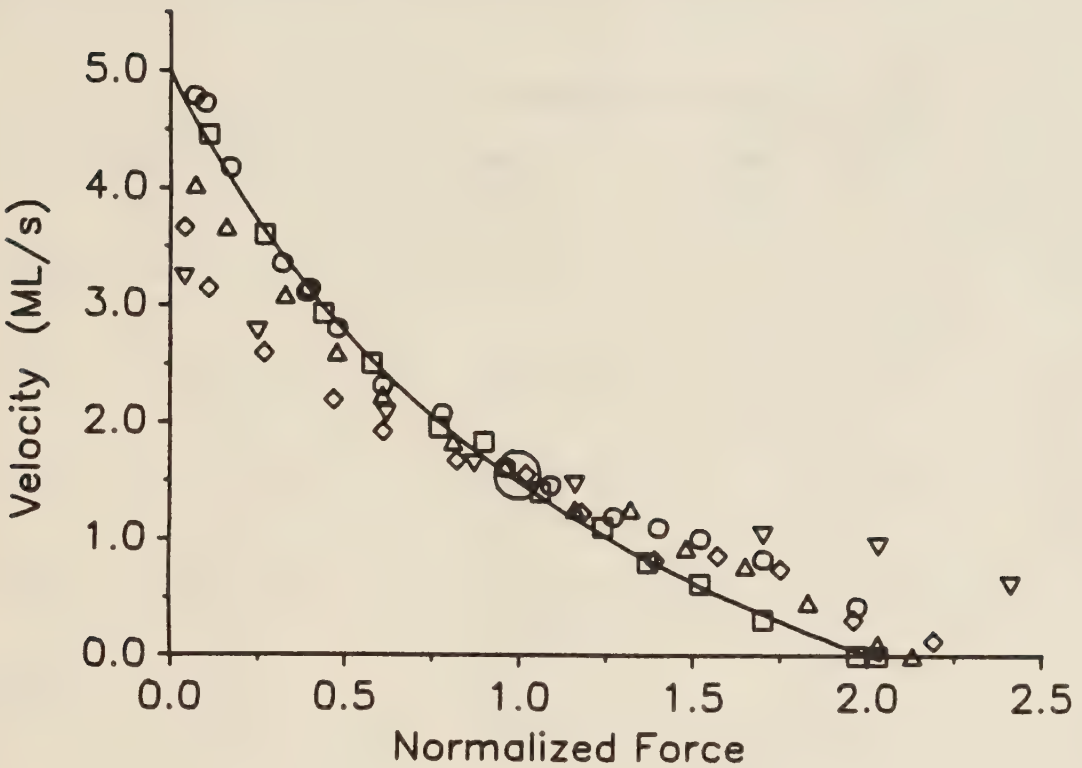

Fig. 3.5. Shortening velocities as a function of the force at a velocity of 1.6 ML/s (circled). This was the velocity at 0.5 Po for this preparation. Data have been replotted from Fig. 3.4, and the same symbols are used. The normalized force of 1 ranged from 17.5 N/cm^2 (0.5 Po) to 2.1 N/cm^2 during the twitch at 2 RT. The curve is the best-fit hyperbola for the non-truncated tetanic data set.

Table 3.1. Force-velocity relations in twitch and tetanic contractions, mean (standard error), n = 5.

		Parameters from the Hill Hyperbola		
	Maximum Isometric force (N/cm^2)	P^*_A (N/cm^2)	Maximum velocity (ML/s)	Curvature (a'/P^*_A)
0.5 RT	28.6 **	32.1 *	5.2	0.46
	(0.4)	(0.1)	(0.1)	(0.03)
1.0 RT	22.8 **	26.3 **	4.7	0.56
	(0.5)	(0.7)	(0.1)	(0.05)
1.5 RT	17.5 **	20.7 **	3.9 **	0.47
	(1.7)	(0.9)	(0.1)	(0.08)
2.0 RT	10.5 **	12.2 **	3.6 **	0.38
	(2.1)	(1.0)	(0.2)	(0.09)
Tetanic	36.3	37.9	5.2	0.62
	(1.4)	(0.9)	(0.1)	(0.07)

RT (rise time) = the time from tension onset to tension peak in the isometric twitch.
Note that the average curvature for the 5 preparations considered separately (0.62) was slightly different from that obtained when all values were combined (0.58, Fig. 3.2).
*,** Student's t-test, paired samples compared to the tetanic value. Statistically significant:
* $p<.01$, ** $p<.001$.

the force axis as the twitch proceeds. The force-velocity curves throughout much of a twitch differ only in the scaling along the force axis. The displacement of the twitch force-velocity curves -- the extent to which they approach that of a tetanic contraction -- is a measure of the state of activation of the muscle. Hill (1949), in his well known investigation of 'active state', chose what I have termed P_A, the maximum force that a muscle can bear without lengthening, as a measure of the position of the force-velocity curve and the state of muscle activation. P_A is a difficult parameter to measure. The force-velocity curves are not steep near the zero-velocity axis and it is not easy to measure with precision the force at which the velocity is zero. Edman (1988) reported that shortening velocity of frog single muscle fibers changed by only 2 % of Vmax over the force range 0.9 to 1.2 Po. In addition, determining P_A requires subjecting the muscle to a series of releases and stretches at moderately heavy loads which can fatigue the muscle and be damaging to it. The definition of the active state was extended by Hill (1951) to include the relative velocity of shortening at a light load, and was used in his investigation of the changes accompanying the transition from rest to activity. The concept was later applied in part of a study of the mechanical properties of a frog sartorious throughout a twitch (Jewell & Wilkie, 1960), and more recently in a study of single frog fiber dynamics during a twitch (Haugen, 1987).

The following section describes how the shortening velocity at a given low load varies throughout the twitch of the locust muscle.

The shortening velocity at different times during a twitch was determined by varying the delay to release from length clamp to force clamp in a series of twitches such that the twitch time course was sampled at 2-4 ms intervals (Fig. 3.6). Force clamps ranged from 0.12 Po to 0.15 Po in the different preparations. By appropriate adjustment of the servo feedback control it was possible to achieve force clamps that varied by no more than +- 5% through the series in any single preparation despite the changing force level at the release and the changing state of activation of the muscle.

Early in a twitch the muscle shortening velocity was nearly the same as that measured during the plateau of a tetanic contraction (Fig. 3.7A). At least briefly the muscle was nearly fully active during the twitch. The velocity values in Fig. 3.7A are plotted as relative velocity (= the velocity during the twitch divided by the velocity at the same load during a tetanic contraction). After a short plateau the relative velocity decreased as a monotonic function of time until late in the twitch (Fig. 3.7A). The relative velocity declined more rapidly the greater the load. This relationship is to be expected, because the maximum load at which the muscle can shorten

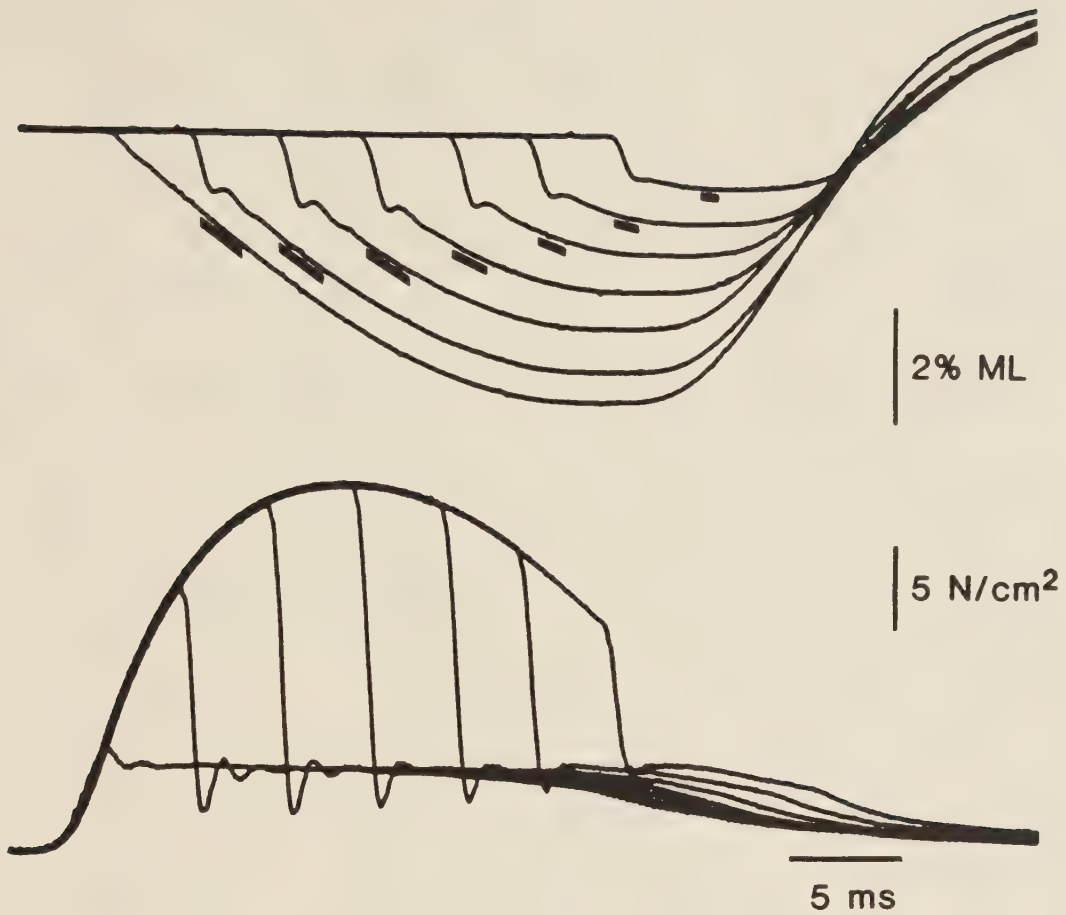

Fig. 3.6. Isotonic shortening at different times during the twitch. The load was clamped to 30 mN (0.15Po) at progressively longer intervals after the stimulus. The thick line just below each position trace marks the period over which velocity and force were measured. The load clamp became increasingly inadequate with increasing time following release because the changing muscle activity changed the optimal feedback parameters, and the latter had been set for best performance shortly after the release.

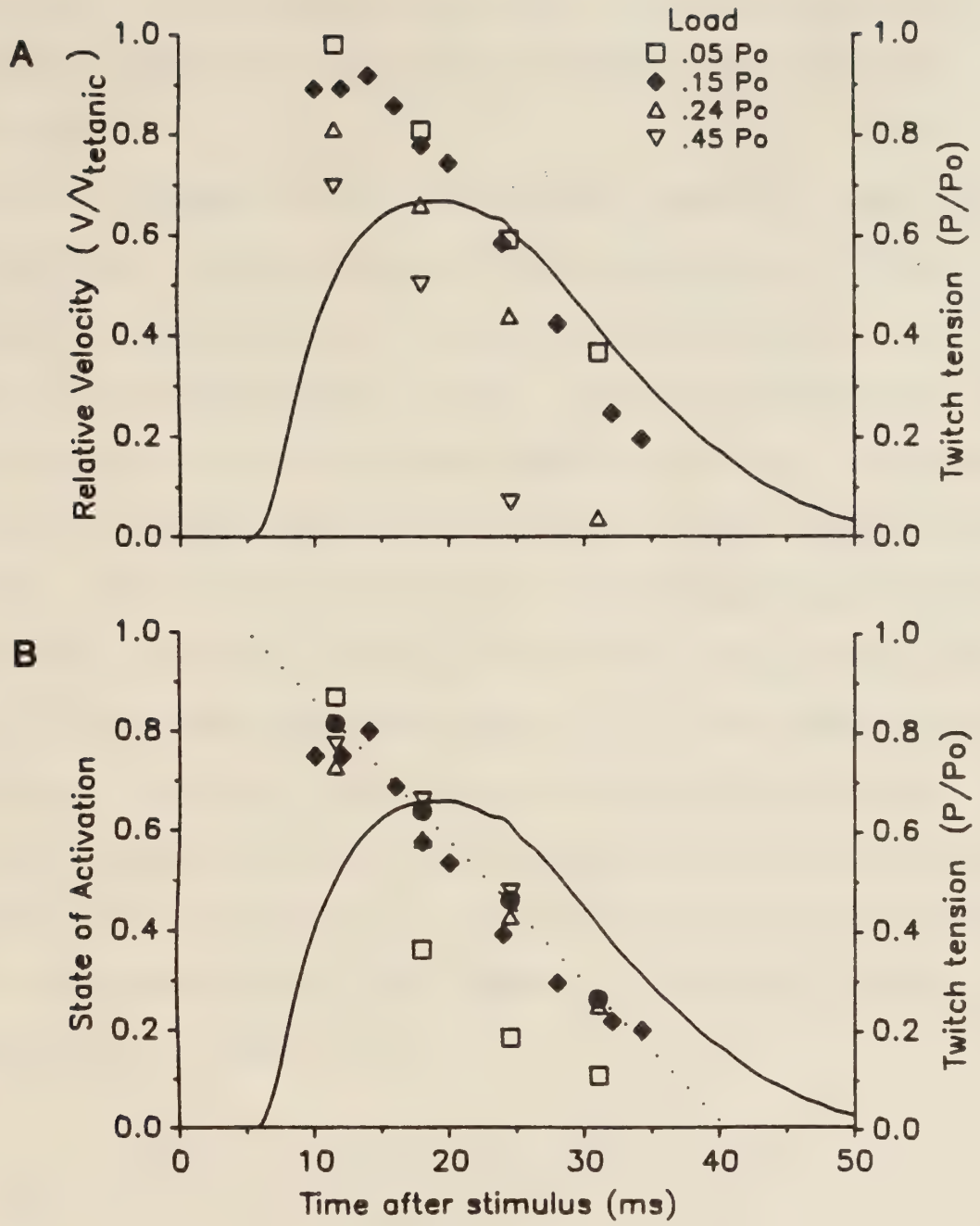

Fig. 3.7. A. Relative velocities during the twitch at different muscle loads. The velocities at 0.15Po were measured from a series of releases as in Fig. 3.6, and reflect velocities at 2-4 ms intervals throughout the twitch. The velocities at heavier and lighter loads were measured during the force-velocity series. In some cases, these velocities were interpolated from the nearest measured velocities.
B. Relative velocities adjusted for the load of isotonic contraction. State of activation is calculated as in the Appendix. The dotted line is a linear regression through the points marked by solid circles, which were directly determined isometric forces (P_A). In each plot, the force in an isometric twitch (P/Po) is included as reference.

declines as twitch activation wanes, and the shortening velocity becomes zero earlier for heavy loads than for light ones.

For two reasons relative velocity <u>per se</u> is not an ideal measure of the state of muscle activation. First, the relative velocity is a function of the muscle load at which it is measured (Fig. 3.7A). Second, the relative shortening velocity is a non-linear function of P_A, the isometric force of which a muscle is capable at any instant (see appendix), and it is P_A that has historically been used to estimate the state of muscle activation. An appropriate measure of the state of activation can be obtained by considering the relative shortening velocity, the load at which it is measured, and the curvature of the underlying force-velocity relationship. The following expression for the state of activation (SA), based on the Hill hyperbola, is derived in the appendix:

$$SA = \frac{L*[\ 1 + (V/Vtet)*(1 - L)/(L + C)]}{1 - C*[(V/Vtet)*(1 - L)/(L + C)]}$$

where $L = P/P^*o$ and $C = a/P^*o$

Calculated values for SA, based on the relative velocities of Fig. 3.7A, are plotted in Fig. 3.7B. With the exception of those points based on very light loads (0.05 Po), the calculated values of SA as a function of time during the twitch are quite similar to one another, and are satisfactorily similar to directly-determined P_A. The

values of SA based on the lightest load tested are distinctly lower than those based on higher loads. This difference may be a consequence of changes in Vmax during the twitch, which is likely to have a proportionally greater effect on the high shortening velocities of low loads than on the slower velocities, that occur at higher loads.

The load range selected for measuring SA, about 0.15 Po, was chosen as one which is high enough to avoid the major problems encountered with very light loads, yet low enough to allow measuring SA to low levels. [It should be noted that in the derivation of SA the relative load, L, is a proportion of the zero-velocity intercept, P^*o. For reasons of convenience, L used in the calculations of SA in this paper were based on measured tetanic tension, Po. P^*o averaged 5% more than Po, thus the effect on L= 0.15 was less than the precision of our calculations. It should also be noted that the derivation of SA may not apply when the L is greater than SA so that the muscle is lengthend by the load rather than shortening against it.]

The earliest time at which relative shortening velocities could be determined in a single twitch was about 10 ms after the stimulus, which is almost half-way through the rising phase of an isometric contraction. At this time the SA was about 0.8 (mean=0.76, s.d.=0.05, n=8, see Fig. 3.8). There was an initial, brief plateau in the SA followed by an approximately exponential decay of SA. A least squares regression line was fitted to log transformed values of SA

for individual preparations. All values of r were 0.99 or higher. The average decay time constant was 14.4 ms (s.d.=1.7, n=5). By 18 ms after the stimulus, which corresponds to the peak of the isometric twitch, SA had declined to about 0.6.

The time course of activation during paired twitches

Because of imperfections in the force control apparatus and because of transient responses inherent to the muscle, stable shortening velocities could not be measured at times much shorter than about 5 ms after the onset of the load clamp (Fig. 3.3A). Further, it was not possible to initiate the load clamp before the onset of muscle tension. At earlier times the muscle was quite compliant, and to obtain even low forces it would be necessary to stretch the muscle very rapidly. In practice the earliest time at which SA could be measured was about 5 ms after the onset of a twitch. By 5 ms after force onset the SA is already high (Fig. 3.8). In order to examine more closely the early time course of SA following stimulation, SA shortening velocities were measured as a function of time in the second twitch of a pair separated by about 28 ms. The timing of the two twitches was chosen such that the force from the first was close to the desired load (about 0.15 Po) at the onset of the second contraction. Because of the residual force from the first twitch, it was possible to initiate load clamps very soon after the stimulus that evoked

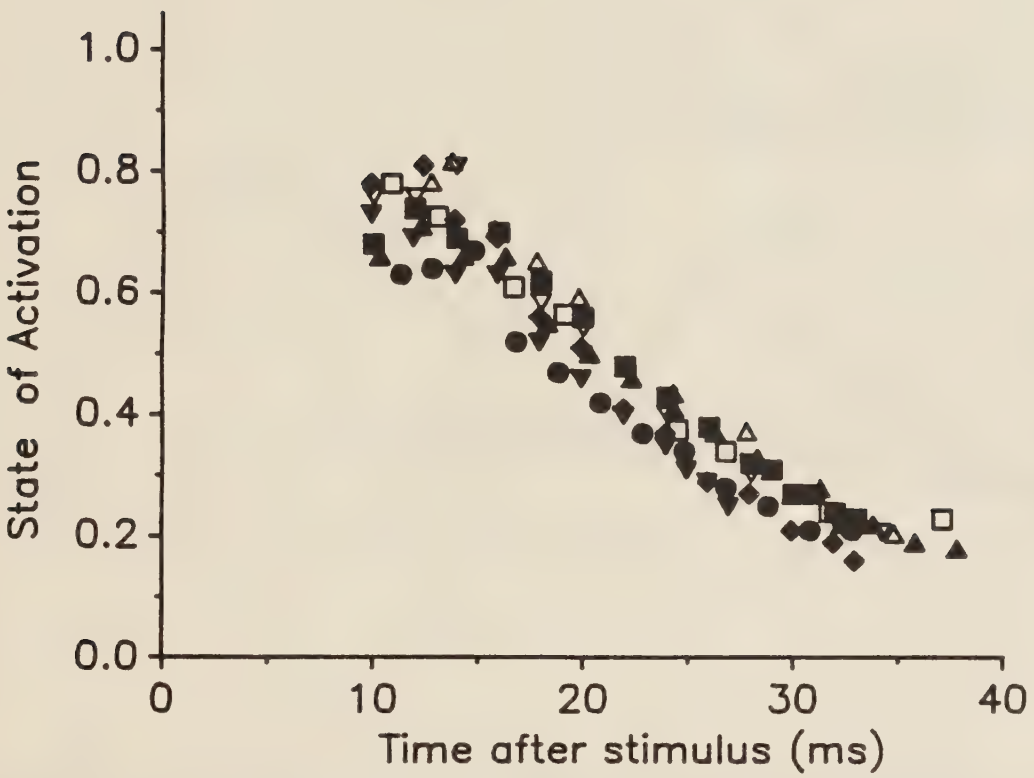

Fig. 3.8. State of Activation (SA) during twitches from 8 preparations. Each preparation is indicated by a different symbol. Force development during an isometric twitch starts about 5 ms after the stimulus.

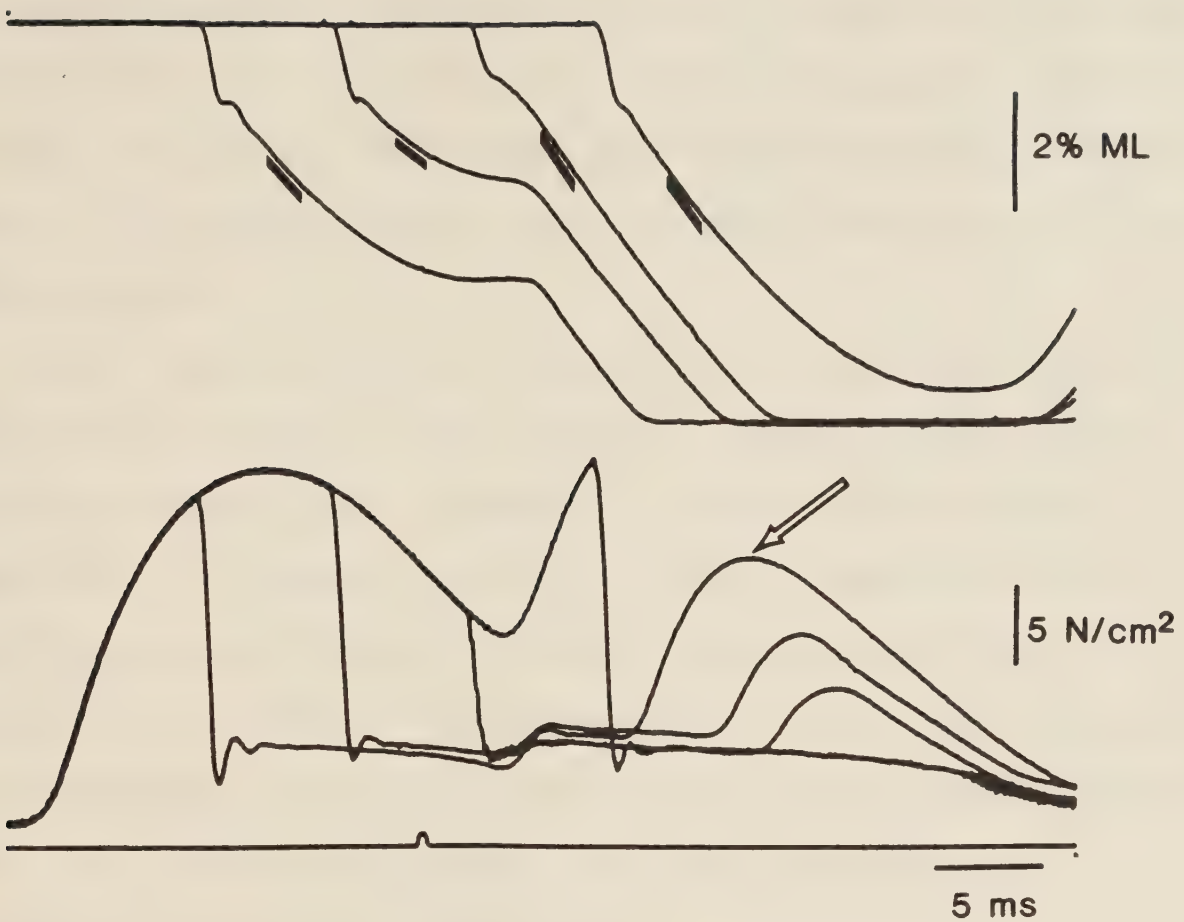

Fig. 3.9. Isotonic shortening during load clamps imposed at different times during 2 partially fused twitches.
The upper set of traces shows position (shortening downward), the middle set of traces shows force. The second stimulus occurs at the time of the upward deflection in the lower trace, which shows the stimulus monitor.
The thick line next to each position trace marks the time over which velocity and force were measured. The arrow marks the isometric redevelopment of force after the earliest release. This force is the 'active state force' as measured by Ritchie (1954).

the second twitch (Fig. 3.9). Values for SA were determined for 1 ms intervals early in the second twitch and at 2 ms intervals thereafter. With all preparations a series of measurements at increasingly longer delay to force clamp was followed by a second series with increasingly shorter delays. The points from both series were similar, indicating no progressive muscle fatigue during the course of the measurements.

The second twitch of a pair is not a perfect model of twitches in general. With closely spaced twitches there is, as already noted, a mechanical summation of forces. In addition, the second twitch of a pair is somewhat potentiated so that the force reached is greater than would be expected from simple summation (Fig. 3.10). The values of SA determined during the rise and fall of the second twitch of a pair should be regarded as values pertaining to a somewhat potentiated contraction.

SA rises abruptly during the second twitch of a pair to a value indistinguishable from that during a tetanic contraction (mean=1.06, s.d.=0.09, n= 5). The SA reaches its peak value less than 3 ms after the time of force onset for the second twitch (Fig. 3.11A). After a brief plateau, SA decays approximately exponentially from its peak value with a time constant of 13.7 ms (s.d.=0.004, n=5), which is not significantly different from that of the first twitch. Potentiation had increased the magnitude of SA, but had not changed the time course of SA decay.

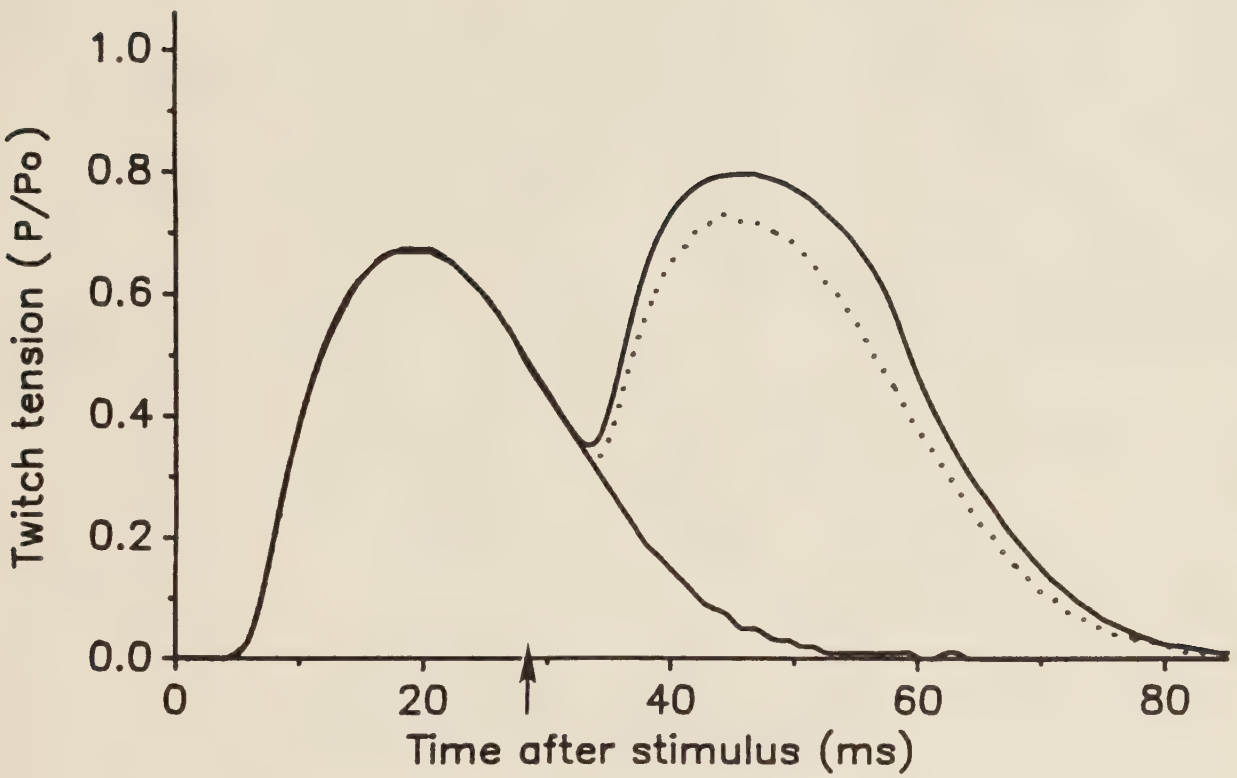

Fig. 3.10. Isometric tension during single and paired twitches. The solid lines superimpose the force traces during a single twitch and during a paired twitch. The time of the stimulus for the second twitch is marked by the arrow. The dotted line is the force from the first twitch alone shifted in time by the interval between the paired stimuli and added to the residual force of the first twitch. Thus the dotted line indicates the force trace that would be expected from simple mechanical summation of the first and second twitches of the pair.

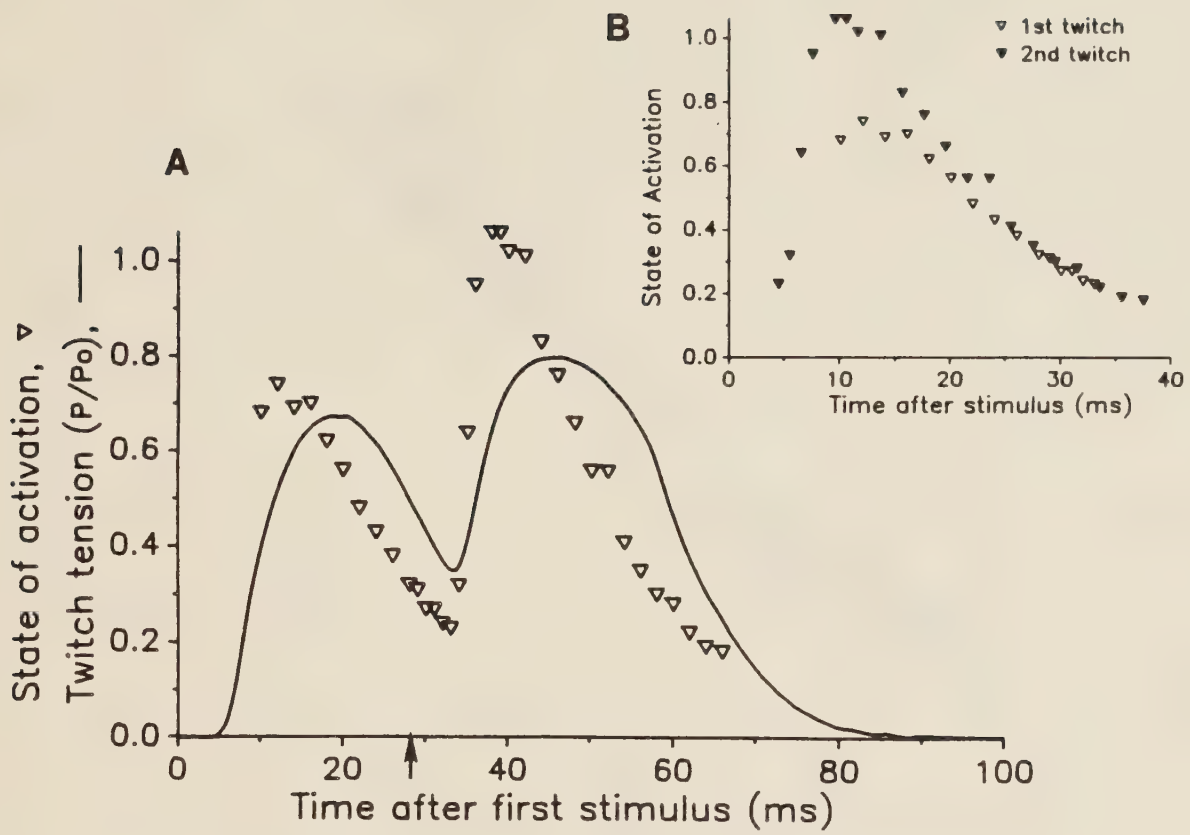

Fig. 3.11. A. Isometric force development and state of activation in paired twitches. The arrow marks the time of the second stimulus.
B. The insert has re-plotted each twitch SA against the time after the last stimulus, so that the 2 twitches may be compared. The falling limbs of the SA curves are identical.

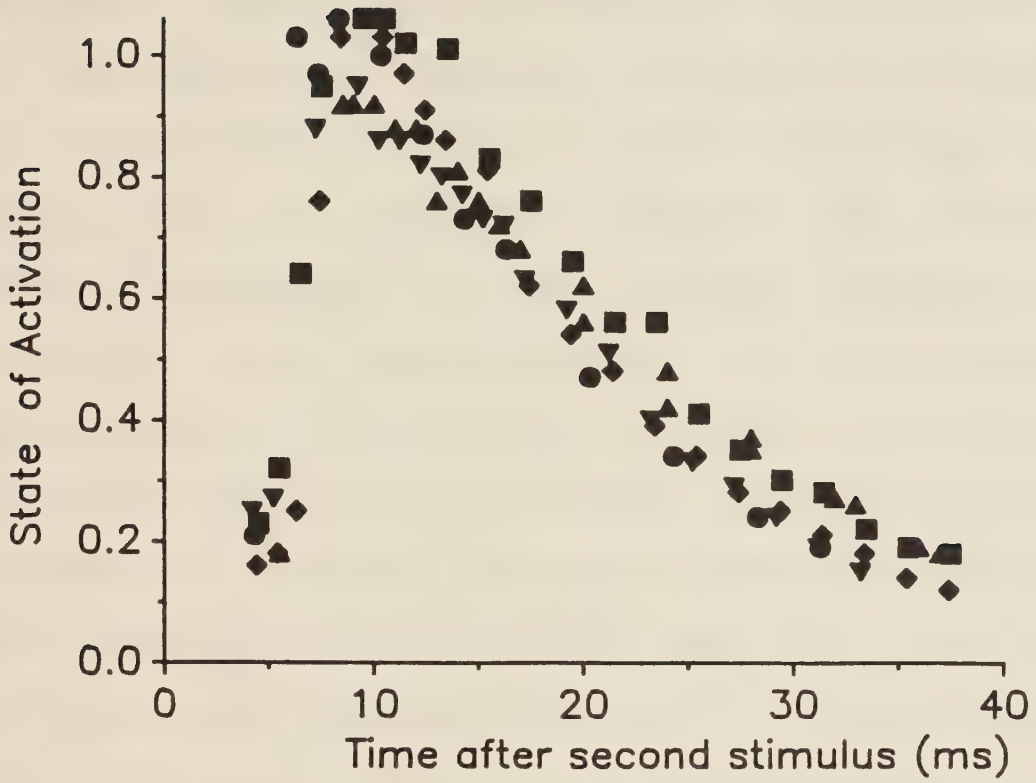

Fig. 3.12. The rise and decline of SA for the second twitch of a pair, determined as for Fig. 3.11. Values are shown for 5 preparations, each indicated by a different symbol. A least-squares regression line fitted to log-transformed values for times greater than 10 ms gave a decay time constant of 14 ms (r =0.98).

SA and other measures of 'active state'

Active state, in the original sense that this term was used (Hill, 1938), is usually considered in the context of a 2 component model of active muscle, one component being the contractile component and the other a series elastic component. Active state is measured as the maximum force that the contractile component can bear without lengthening. This is the force on the whole muscle under conditions in which the contractile component is isometric. The contractile component is isometric when the muscle length is constant (i.e., when dML/dt = 0) only if the muscle force is also constant (dP/dt = 0), for it is only at constant force that the series elastic component neither lengthens nor shortens. One way to determine the active state force is to determine that load in a force clamp at which muscle shortening velocity is 0. Values for active state determined in this way are plotted in Fig. 3.7B, and there is good agreement between these measures and those of SA.

The conditions under which muscle force equals active state force, dML/dt = 0 and dP/dt = 0, are also met at peaks and valleys of the force trace during isometric contraction. Ritchie (1954) used the time and amplitude of the force peak reached during tension redevelopment following a quick release to chart the decline of active state late in the twitch. Edman (1970) imposed a quick release during a short unfused tetanus, varying the time of release

after the stimulus. The rise time of the active state was determined during isometric force redevelopment from the minimum force reached between contractions. The decay of the active state was determined as in Ritchie's quick releases. Using an approach analogous to that of Edman, I determined the rise time of the active state from the time and amplitude of the tension minimum between a pair of twitches at varied stimulus intervals (Fig. 3.13A). In addition, the delay and amplitude of the peak of the second twitch gave information on the early portion of active state decay. As the interstimulus interval of the twitches was shortened, the second twitch peak was higher, as also was the intersection force between the two twitches.

Finally, in the load clamp experiments with double twitches, since the load was low contraction speed was high if the release to isotonic contraction came early in the twitch. Thus, after early releases the muscle shortening was halted by a pre-set stop and the muscle contracted isometrically after the stop was reached (Fig. 3.9). The time and amplitude of the redeveloped tension during isometric contraction against the stop measures the later portions of the decay of active state in the same manner as did Ritchie (1954).

The time course of the active state for the second twitch of a pair, determined from tension maxima and minima during isometric contraction, is compared with SA values from the same preparation, in Fig. 3.14. There is good

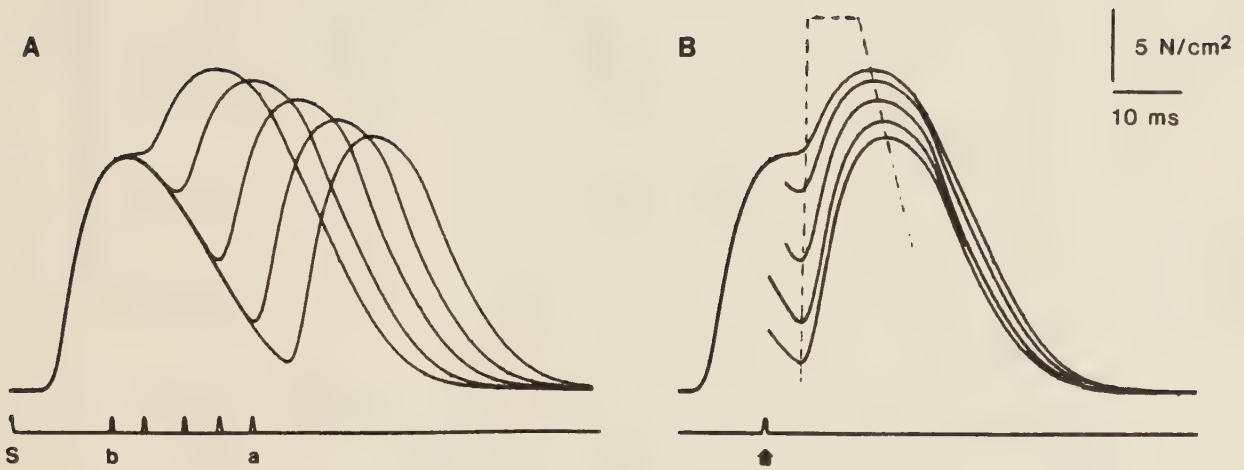

Fig. 3.13. Progressive fusion of twitch pairs.
The top sets of traces show force, and the bottom traces show the stimulus monitor.
A. Several twitch pairs are shown. In each, the first stimulus (S) is followed by a second stimulus, at varying intervals: from one (a) at an interval long enough so that there is little second twitch potentiation, to one (b) at a time that has allowed no force relaxation in the first twitch.
B. The force traces have been overlayed so the 2nd stimulus of each pair coincide (arrow). The dotted lines show an approximation of the rising and falling phases of the active state, as defined by Hill (1949).

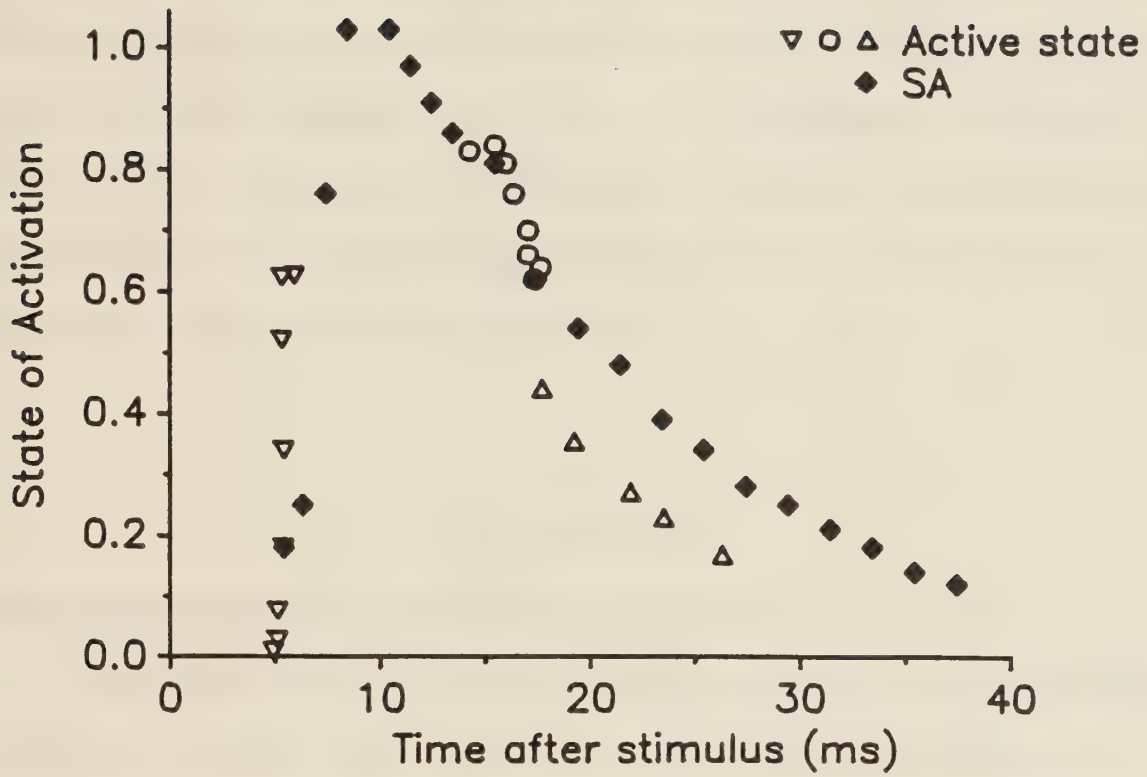

Fig. 3.14. The rise and fall of muscle activation as measured in Figs. 3.9 (▽ , o) and 3.13 (△) during isometric contractions (active state), compared to that as measured in Fig. 3.6 during isotonic contractions (SA).

agreement between SA and active state determined from purely isometric contraction of twitch pairs. There is obvious divergence between SA and active state measured by the redevelopment of tension following an isotonic contraction. The reason for the difference between SA and active state measured from tension redevelopment is not certain. It is possible that there was shortening inactivation during the isotonic portion of the contraction preceding the tension redevelopment (see Fig. 8, Josephson & Stokes, 1989) which depressed the degree of muscle activation and resulted in the difference between SA and active state measured with Ritchie's approach.

DISCUSSION

The changing state of activation during a twitch

The relationship between muscle force and shortening velocity changes systematically during the course of a twitch. Early in the twitch the force-velocity relationship is quite similar to that during the plateau of a tetanic contraction. As the twitch proceeds the maximum isometric force progressively declines, as does the shortening velocity at any force less than the maximum value (Figs. 3.4, 3.15). I have quantified the change in the position of force-velocity curves throughout a twitch with a parameter termed state of activation (SA). SA predicts the relative force at the intercept of the force-velocity

curve and the zero velocity axis; this force is close to but not identical to the actual maximum force that the muscle can sustain without lengthening. SA is derived from the shortening velocity at a moderately low load relative to the shortening velocity at the same load during a tetanic contraction. The derivation of SA is based on 3 assumptions: (1) that force-velocity relationships are reasonably represented by a rectangular hyperbola as proposed by Hill (1938); (2) that the maximum shortening velocity (= intercept of the hyperbola with the zero force axis) does not change through a twitch; and (3) that the curvature of the hyperbola (= a/P^*_A) does not change during the twitch. The latter two assumptions are reasonably valid early in the twitch but become increasingly less defensible as the twitch progresses. I have not examined in detail the extent to which SA will be affected by changes in curvature and maximum shortening velocity, but the practical effects of changes in these two parameters appear to be small since SA does predict quite well the maximum force that the muscle can bear to times quite late in the twitch (Fig. 3.7B). The changing force-velocity relationships for a muscle throughout the rise and decay of a twitch, predicted from measured values of SA, are plotted as a velocity surface in Fig. 3.16. The shortening velocities are high at low loads and early in the twitch, but diminish both in the direction of heavier loads or, after the plateau of activation, later during the twitch.

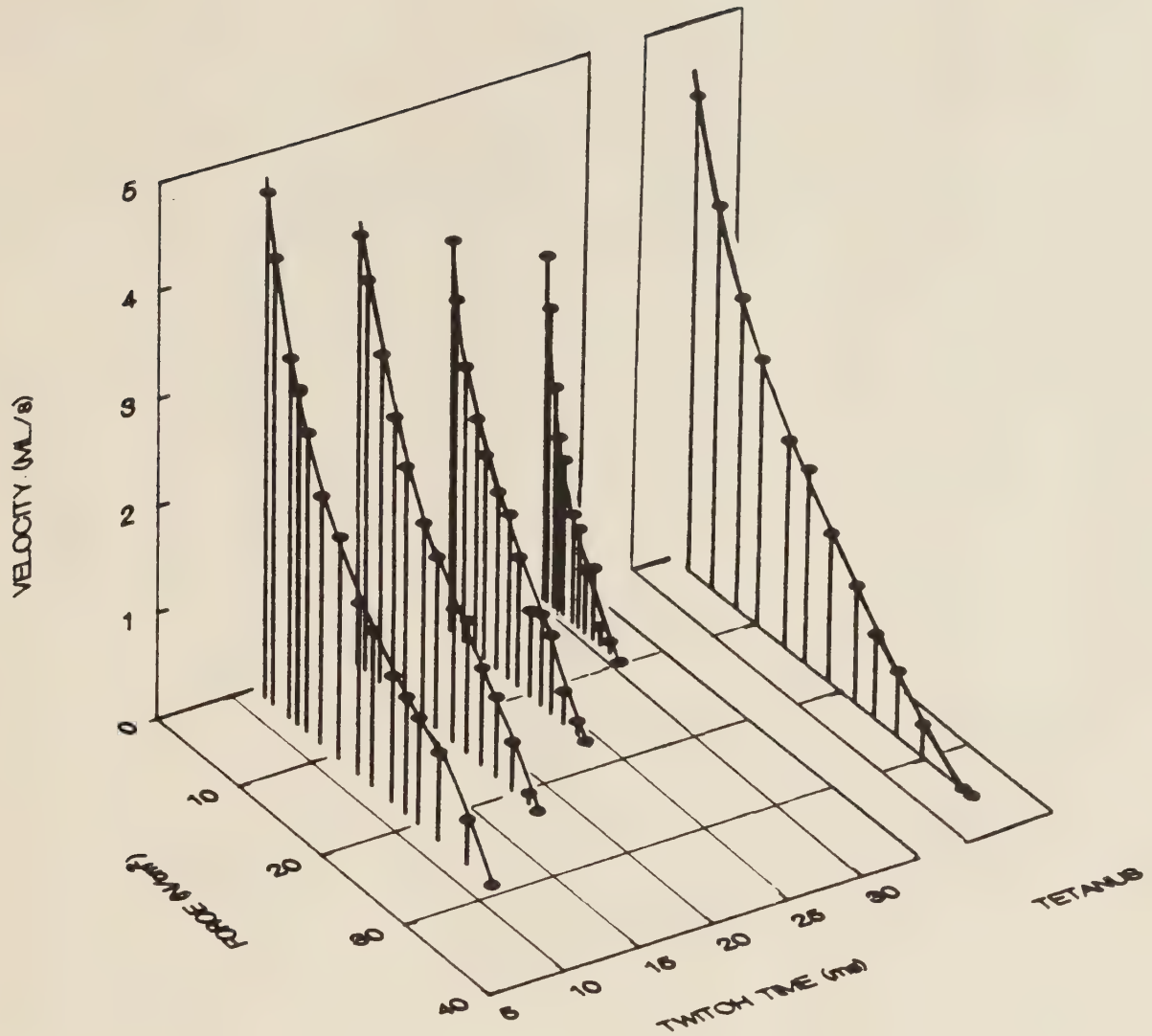

Fig. 3.15. Force-velocity data from isotonic load clamps during a twitch.
The data from Fig. 3.4 have been replotted so that the decrease in velocities at various loads can be seen as a function of time. The stimulus was at time 0. Velocities during a tetanic contraction are plotted at the right for comparison. In this muscle the latency to force onset was 5.1 ms, the isometric twitch rise time 13 ms, and the decay time from twitch peak to 0.5 relaxation was another 13 ms. Each velocity is connected by a line to the corresponding time and force at which it was measured. The isochronic force-velocity curves connecting the points were fitted by eye. Compare these force-velocity curves with those predicted from SA in Fig. 3.16.

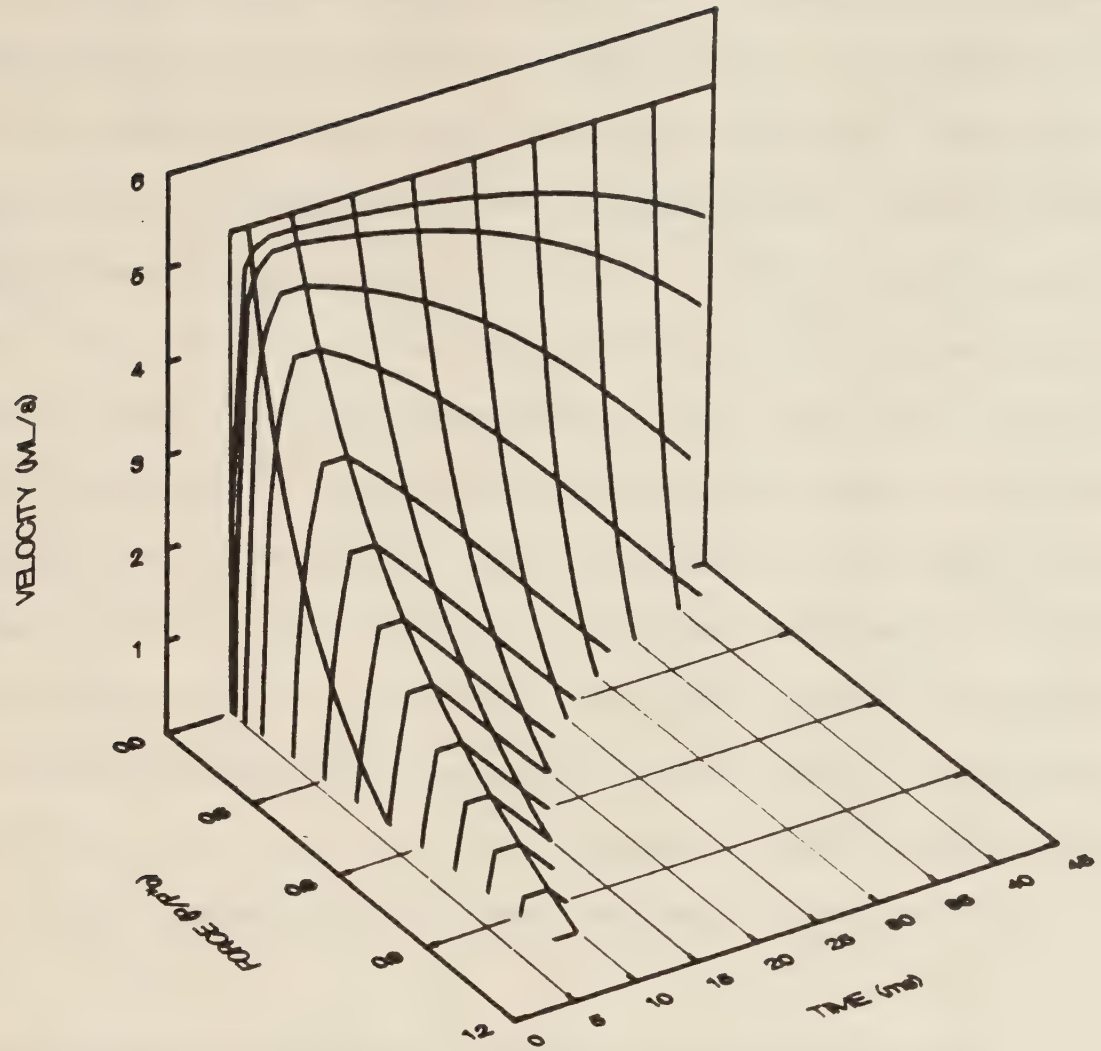

Fig. 3.16. Projected velocity surface during a twitch, plotted as a function of force and time (ms after stimulation). Velocities were calculated from a modified Hill Hyperbola: $V = b*(SA - L)/(L + C*SA)$ where L is the relative load (= P/P^*o), C is the curvature (= a/P^*o); P^*o, a and b were taken from the grouped tetanic data of Fig. 3.2. The time course of SA was based on that shown in Fig. 3.12, which was derived for the second of paired twitches. It is assumed that SA is 0 for a latent period of 5 ms after the stimulus, it then rises linearly for 3 ms to reach 1, remains at 1 through a 2 ms plateau, then decays exponentially with a time constant of 14 ms. Full force-velocity curves were calculated for one-half way through the twitch rise time (6.5 ms), for the end of the SA plateau (10 ms), and at 5 ms intervals thereafter. Velocities were calculated at loads of 0, 0.01 P^*o, $0.02P^*o$, 0.05 P^*o, 0.1 P^*o, and at 0.1 P^*o intervals thereafter throughout the twitch.

The general shape of the time course of SA in the locust muscle is similar to that of the time course of active state reported for frog muscle (Hill, 1949), with a rapid rise, a plateau (brief in the locust muscle), and an approximately exponential decay. In the locust muscle the rise time of SA (2-3 ms) was about 20% of the isometric twitch rise time. In frog muscle the rise time of the active state has been reported as being about 10% of the twitch rise time (Hill, 1951; estimated from Fig. 3.1 in Edman, 1970). On the other hand the relative shortening velocity for a frog muscle fiber was recently reported to increase through the first half of the twitch rise time (see Fig. 3.4, Haugen, 1987; Haugen & Sten-Knudsen, 1987), implying a slower development of muscle activation.

The maximum velocity of shortening during a twitch

There is an unresolved controversy about whether the maximum velocity of shortening (Vmax) varies with the state of activation of muscle. Jewell and Wilkie (1960) found that Vmax decreased progressively through the twitch of a whole frog sartorius, as did the isometric force. In contrast, Edman (1979) found that Vmax in single fibers was the same during the peak of a twitch as during the plateau of a tetanic contraction, even though the states of activation of the muscle are presumably different at these times. Haugen (1987) also reported no difference in Vmax at times

from early in the rising phase of twitch contraction well into the relaxation phase.

Reports are also inconsistent about whether Vmax varies with changing states of activation created by changes in cytoplasmic calcium concentration in skinned muscle fibers (Julian et al. 1986; Podolin & Ford, 1986). It has recently been observed that at moderate calcium levels (0.6 maximal activation and above) Vmax is calcium insensitive for short times and shortening distances at zero load, but calcium sensitive for longer times and shortening distances (Farrow et al. 1988). At lower calcium levels Vmax was found to be calcium sensitive at all times. This group has proposed that calcium serves only as a switch for the early, transient velocity, removing the inhibition from the cross-bridge sites, but that the steady-state velocity of unloaded shortening is linearly proportional to the level of active tension.

In the locust muscle, I found previously that a moderate increase of isometric tetanic tension, brought about by the neurohormone octopamine, did not produce a concomittant increase in Vmax (Malamud et al. 1988). In the present experiments, a moderate decrease in the state of activation also did not change the maximum velocity, although larger decreases in SA did. Midway through the twitch rise time, when SA had decreased by 20%, Vmax was as high as that in a fully developed tetanus. There was, however, an obvious and significant reduction in Vmax later in the twitch

(Table 3.1). It might be noted that there is a very steep increase in shortening velocity with decreasing load in the low force, low activation region of the velocity-force-time surface (Fig. 3.16). At low levels of activation a small, systematic error in the measurement of muscle force could lead to a systematic error in the estimate of Vmax (see also Pate & Cooke, 1987). While I have no reason to believe that the progressive decrease in calculated Vmax late in a twitch is a consequence of a systematic measurement error, this possibility can not be rigorously ruled out.

The time course of SA and force development during a twitch

When two twitches are evoked at a short interval the tension reached during the second twitch can be greater than that at the peak of the first, both because of mechanical summation and because the second twitch becomes potentiated by the preceeding activity (Fig. 3.10). Early in a potentiated twitch the value of SA is 1; the shortening velocity of the muscle against a given load is indistinguishable from that during the plateau of a tetanic contraction. For technical reasons it was not possible to measure SA very early in single twitches, but at the earliest times at which it could be measured in a single twitch SA was less than that in a potentiated twitch (Fig. 3.11). From this result, I conclude that SA does not reach 1 during a single twitch. This conclusion is in contrast to

results from frog muscle in which it is reported that the muscle does become fully activated even during a single twitch (Hill, 1949; but see Haugen & Sten-Knudsen, 1987). By analogy with other insect flight muscles, it is likely that the locust flight muscle is multiterminally-innervated, and that the electrical event of excitation-contraction coupling is a distributed synaptic potential rather than a propagated action potential (Josephson & Stokes, 1982). Synaptic potentials are graded rather than all-or-nothing, and it is possibly because of synaptic facilitation and increasing muscle fiber depolarization, that SA reaches higher values in the second twitch of a pair than during the first.

As is expected from the two-component model of muscle used by Hill (1938), the time course of SA rise and fall is briefer than that of tension rise and fall during an isometric twitch (Figs. 3.3, 3.7B). The isometric force after the peak of the twitch is greater than the force that the muscle can just sustain because, in fact, during the relaxation phase of an isometric contraction the contractile element is not isometric, but is lengthening (Hoyle, 1983). The slope of the force-velocity curve near Po is very shallow (Edman, 1988), so even slow lengthening velocities are resisted with forces substantially greater than the isometric force -- leading to muscle force during 'isometric relaxation' which is significantly higher than the force determined from SA.

SUMMARY

The force-velocity relationships during isotonic shortening of the tetanically stimulated Tcx_2 muscle of the locust, S. Americana, were fit to a Hill hyperbola. The maximum shortening velocity (Vmax) determined from the force-velocity curve was 5.2 ML/s (25 ^{o}C) and the curvature ($a/P^{*}o$) was 0.62. The maximum isometric force was 36.3 N/cm^2.

Force-velocity curves were determined by release to isotonic shortening at different times during twitch contractions. Early in a twitch (at times shorter then the isometric twitch rise time) the values for Vmax and curvature were similar to those measured during tetanic contractions, but the intercept with the force axis was less. The force-axis intercept was progressively displaced with time toward lower values. Later in the twitch Vmax declined.

A parameter termed State of Activation (SA) is proposed as a measure of the force generating capacity of a muscle during isotonic contractions. SA is determined from the shortening velocity at an intermediate load, and is the predicted intercept of the force-velocity curve with the force axis relative to that which pertains during a tetanic contraction.

SA was determined for single twitches and for paired twitches. With paired twitches it was possible to determine the rise time of SA as well as its decay.

At the end of the latent period the SA rises in 2-3 ms to a maximum. The peak value of SA during a single twitch is about 80% of the tetanic value; during the second twitch of a pair (interstimulus interval = 28 ms) the peak value of SA is similar to that during tetanic stimulation. After a brief plateau SA declines approximately exponentially. The time constant of decay is about 14 ms at 25 ^{o}C.

APPENDIX

PREDICTING THE INTERCEPT WITH THE FORCE AXIS OF A FORCE-VELOCITY CURVE FROM THE VELOCITY MEASURED AT A SINGLE FORCE

(Contributed by R.K. Josephson)

Assume that at any time during a twitch or tetanus the force-velocity relationships are described by a Hill hyperbola:

(1) $(P + a')*(V + b') = K = (P^*_A + a')*b'$

P = force on muscle
V = shortening velocity
P^*_A = zero-velocity intercept with force axis
a', b' are parameters reflecting the position of the asymptotes of the hyberbola.
a', b', and P^*_A are constant for a muscle at a given time, but each may change with state of muscle activation.

The following form of equation (1) will be used for the special case of force-velocity relationships during the plateau of a tetanic contraction:

(2) $(P + a)*(V_{tet} + b) = (P^*o + a)*b$

Solving (1) for velocity gives:

(3) $$V = b'* \frac{P^*_A - P}{P + a'}$$

The maximum shortening velocity, V_{max}, is achieved when P = 0. Setting P = 0 in (1) and solving for b' gives:

(4) $$b' = \frac{a'}{P^*_A} *V_{max}$$

The following definitions will be used:

(5) L = relative load = P/P^*o
(6) SA = state of activation = P^*A/P^*o
(7) C = curvature of force-velocity hyperbola = a/P^*o

It should be noted that SA measures the zero velocity intercept of the force-velocity curve relative to that during a tetanic contraction. It is a measure of the position of the force-velocity curve along the force axis, essentially how the curve is scaled on the force axis. SA will be used as the measure of the extent of muscle activation.

Force-velocity curves through at least the early part of a twitch differ substantially only in their position along the force axis. They have the same maximum shortening velocity, Vmax, and the same curvature, a'/P^*_A (Fig. 3.5, Table 3.1). The following derivation applies exactly for those conditions when Vmax and a'/P^*_A are constant. Then:

(8) $C = a'/P^*_A = a/P^*o$

(9) $b' = b$ (this follows because the two factors in (4) which are used to calculate b are each constants.

Dividing (3) by the equivalent form for a tetanic plateau, re-arranging, and cancelling b and b' as being common factors gives:

$$\frac{V}{V_{tet}} = \frac{(P^*_A - P)*(P + a)}{(P^*o - P)*(P + a')}$$

Making the following substitutions:

$$P^*_A = SA*P^*o$$
$$a' = C*P^*_A$$
$$P = L*P^*o$$

and solving for SA gives:

$$SA = \frac{L*[1 + (V/V_{tet})*(1 - L)/(L + C)]}{1 - C*[(V/V_{tet})*(1 - L)/(L + C)]}$$

Thus if a'/P^*_A and Vmax remain constant, the zero velocity intercept of the force-velocity hyperbola, here indicated by SA, is determinable from: (1) V/V_{tet}, the velocity of shortening at a given load divided by the velocity at the same load during a tetanic contraction; (2) L, the load at which the velocity was measured relative to the maximum tetanic force; and (3) C, the curvature of the force-velocity relationship.

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